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ADVANCES IN CARBOHYDRATE CHEMISTRY

VOLUME 3

ADVANCES IN CARBOHYDRATE CHEMISTRY

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EDITORS' PREFACE

In presenting this third volume in the annual series of Advances in Carbohydrate Chemistry, the editors are pleased that the international scope of their endeavor has been further increased, the present volume containing contributions from England, Scotland, Sweden and Germany in addition to those from the United States. It is a matter of satisfaction to note that Volume 1 has gone into its second printing. The editors have endeavored to maintain this publication on a high scholarly level and they thank the contributors for their full cooperation in this effort.

Our executive committee has lost the services of Dr. R. Max Goepp, Jr., by his tragic and untimely death in an airplane accident. His enthusiastic support will be greatly missed. We are proud to welcome as new members of the executive committee: Professor E. L. Hirst, of the University of Edinburgh; Dr. R. C. Hockett, scientific director of the Sugar Research Foundation, New York; and Professor C. B. Purves, of McGill University.

Dr. Stanley Peat, of the University of Birmingham, has continued to render assistance as associate editor for the British Isles. Dr. C. S. Hudson has again very materially assisted in the editing of this volume. The subject index has been compiled by Dr. L. T. Capell. Valuable editorial assistance has been rendered by Mr. Donald O. Hoffman.

THE EDITORS
W. W. P.
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RUDOLPH MAXIMILIAN GOEPP, JR.[1]

On October 3, 1946, a New York to Berlin transatlantic plane had completed a refueling stop at a Newfoundland airport. Although the visibility was excellent, the pilot failed to gain altitude on the take-off. The airplane struck a cliffside and disappeared in a sheet of flame. No one survived. Among the passengers were five chemists traveling to Germany, under the auspices of the American government, to investigate chemical developments in that war-devastated land. One of these was Dr. R. Max Goepp, Jr., who had previously spent several months in Germany and was returning to complete his work. In this tragic manner American chemistry lost a promising young leader who was very active in the affairs of the Division of Sugar Chemistry and Technology of the American Chemical Society (Chairman, 1941) and who was a member of the executive committee of Advances in Carbohydrate Chemistry.

Rudolph Maximilian Goepp, Jr., was born April 3, 1907 in the city of Philadelphia. His mother, Josephine Rutter Pyle, was a member of a family that had settled in West Chester, Pennsylvania, before the American Revolution. His father, a physician and formerly professor of theory and practice, Woman's Medical College of Pennsylvania, is a descendant of Philip Henry Goepp who came to Bethlehem, Pennsylvania, in 1834 in the capacity of Administrator of the Moravian Estates and who was a member of a family long resident in Heiligstein near Strasbourg in Alsace. Max graduated from the West Philadelphia High School in 1924 and in 1928 received the B. S. degree from Lehigh University in Bethlehem, Pennsylvania. Here his interest in chemistry was aroused by his professors Ullman, Babasinian and Neville. His scholarship was attested by election to Phi Beta Kappa and Tau Beta Pi. In 1928 he rated the highest score in the State of Pennsylvania in a college achievement test conducted by the Carnegie Foundation for the Advancement of Teaching. After holding the New Jersey Zinc Company research fellowship at Lehigh University in 1928–1929, he was appointed a Rhodes scholar and studied at Oxford University where he was resident at Queens College and received his D. Phil. degree in organic chemistry under F. D. Chattaway in 1932. During this period of European train-

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(1) A photograph of the subject of this sketch appeared in *Advances in Carbohydrate Chem.*, **2**, frontispiece (1946).

ing he attended a summer session at the University of Hamburg and on a visit to Berlin met Miss Elisabeth Wenning who was studying in the German universities as an exchange student from Vanderbilt University of Nashville, Tennessee. They were married October 6, 1934 and to them were born three children: Carla Elisabeth; R. Max Goepp III; and Hildegarde Wenning.

In 1932 Dr. Goepp joined the research staff of the Atlas Powder Company of Wilmington, Delaware, in the capacity of research chemist and in 1944 he was appointed director of organic research, which position he held until his untimely death at the age of 39 years. Here he was closely associated with Mr. K. R. Brown in developing the manufacture of sorbitol and mannitol by the electroreduction of D-glucose under the Creighton patents.[2] Although begun under great difficulties in a period of world depression, this venture prospered and was especially useful in the country's war economy by providing a substitute for glycerol and a raw material for ascorbic acid.

Dr. Goepp was never content with being strictly an industrial chemist. He was ever intrigued by the more academic and theoretical aspects of the complex reactions and substances with which his industry dealt. It was his thesis that intelligent progress in this technology would be dependent largely upon an understanding of the underlying chemistry of the materials involved. To this end he associated with himself at various periods a number of academic investigators: Werner Freudenberg of Fordham University; R. C. Hockett and H. G. Fletcher, Jr. of the Massachusetts Institute of Technology; and the writer of this sketch. In these endeavors Dr. Goepp was a true scientific collaborator, spending much time with conferences and detailed correspondence concerning the various problems and contributing a great deal to their intelligent direction.

Max Goepp was an unusual person. He was six feet, six inches in height and his towering figure presented a striking picture in the crowded hotel corridors of the American Chemical Society meetings. Like most exceptionally intelligent persons, he had a wide range of interests. He was a proficient flutist and composed good poetry for the enjoyment of himself and friends. He and his wife made photographic records of the cabalistic paintings on the big barns of the Pennsylvania Dutch farmers—artistic endeavors which had their origin far back in the traditions of the Palatinate. He was a respected and well-known citizen of the old town and port of New Castle, Delaware. One of the pleasant experiences the

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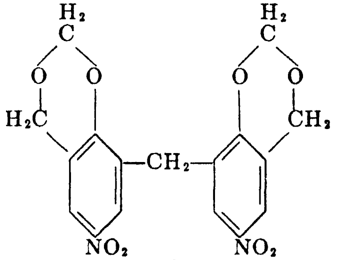
(2) H. J. Creighton, *Trans. Electrochem. Soc.*, **75**, 289 (1939); H. J. Creighton, U. S. Pats. 1,612,361 (1926); 1,653,004 (1927); 1,712,951 (1929); 1,712,952 (1929), 1,990,582 (1935).

writer will long remember was a tour through this town under Max's guidance.

In his dissertation (13),* Goepp contributed to Professor Chattaway's classical organic program by studying the reaction of formaldehyde with *p*-nitrophenol. He determined that bis(6-nitro-1,3-benzodioxan-8-yl) methane (I) was one of the reaction products.



I

Sorbitol[3] is one of the most difficultly crystallizable sugar derivatives. It exhibits a characteristic tendency to separate from solution in the form of fine crystals embedded in an amorphous gel matrix. Emil Fischer and his co-workers[4] never succeeded in preparing a sorbitol of high purity. The most highly purified sorbitol ever reported was that described by Rose and Goepp (15), who also cited revised constants for D-mannitol. Goepp's patent (33) on the crystallization of sorbitol disclosed the first commercial process for preparing crystalline, free flowing sorbitol. Previously, this substance had been sold in 50% aqueous solution; as translucent lumps containing 3–5% water; or as a spray-dried powder. All of these preparations contained the impurities developed during the manufacture. An interesting contribution to hexitol chemistry was the recording of the last of the theoretically possible hexitols, L-talitol (18).

The use of sorbitol in enhancing the clarity of urea-formaldehyde resins is described in a patent (30) as is also its use with inorganic materials to form useful plastics (31). A dianhydride of sorbitol is described as a humectant for tobacco (34). The main industrial studies of Goepp were concerned with the use of sorbitol and D-mannitol in the manufacture of resins of the ester gum (esters with rosin acids) or alkyd (linseed oil-phthalic anhydride-hexitol) types (32, 14). It was shown that in the preparation of these polyesters the behavior of the hexitols is different from that of glycerol in that the hexitols undergo simultaneous inner etherification to form mono or di-anhydrides, thus leaving only four or two hydroxyls available for esterification. The products so formed found a very considerable application during World War II. Corresponding esterification reactions with fatty acids and the hexitols or their anhydrides led to the synthesis of materials suitable for plasticizing vinyl resins (35) or cellulose esters (36).

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* See bibliography on page xxii.

[3] In accordance with a suggestion of Dr. Goepp, "sorbitol" will be employed as a trivial name for D-glucitol.

[4] See E. Fischer and R. Stahel, *Ber.*, **24**, 528, 2144 (1891).

The inner etherification induced in the sorbitol and D-mannitol structures during polyester formation, made a study of their inner ethers or anhydrides a matter of practical significance. A new monoanhydride of sorbitol was described (21) and designated arlitan (from the initials of the Atlas Research Laboratory). Identification as 1,4-anhydro-D-glucitol was made by comparison of its sirupy tetramethyl ether with that obtained by acid anhydrization of Irvine's 2,3,5,6-tetramethyl-D-glucitol[5]; by oxidation of the tetramethyl ether to dimethyl-L-threaric acid [O-dimethyl-L-(*dextro*)-tartaric acid] and identification as the crystalline diamide; and by its behavior on oxidation with lead tetraacetate.[6]

Brigl and Grüner's crystalline anhydride of 1,6-dibenzoyl-D-mannitol[7] (obtained by heating this substance in high-boiling solvents with a small amount of *p*-toluenesulfonic acid) was debenzoylated (24) to the sirupy anhydrohexitol and this was shown to produce no significant quantity of formaldehyde with lead tetraacetate. With the same reagent the dibenzoate yielded a crystalline dialdehyde dihydrate (II) whose optical inactivity eliminated the D-mannitol configuration for the dibenzoylanhydrohexitol and demonstrated that in the oxide ring formation a Walden inversion had occurred on either carbon two or five. Since the D-mannitol molecule possesses an axis of symmetry, these two positions are equivalent and the inactive product could only be derived from the D-glucitol configuration. The 2,5-anhydro-1,6-dibenzoyl-D-"mannitol" of Brigl and Grüner[7] was therefore shown to be 2,5-anhydro-1,6-dibenzoyl-D-glucitol.

Brigl and Grüner[7] reported another dibenzoyl-anhydro-D-mannitol (from the above-described reaction) which was shown (23) to be 1,4-anhydro-D-mannitol 2,6(or 3,6)-dibenzoate. Saponification yielded a crystalline anhydro-D-mannitol (D-mannitan) which was identical with that described previously by Valentin.[8] The 1,4-anhydro-D-mannitol structure was established by the method of synthesis employed by Valentin;

---

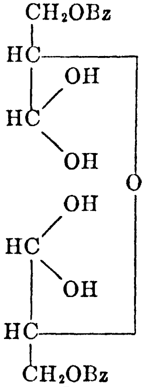
(5) J. C. Irvine, A. W. Fyfe, and T. P. Hogg, *J. Chem. Soc.*, **107**, 524 (1915).

(6) R. C. Hockett, Maryalice Conley, M. Yusem and R. I. Mason, *J. Am. Chem. Soc.*, **68**, 922 (1946).

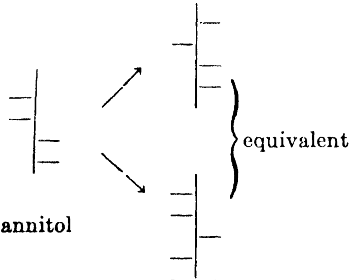
(7) P. Brigl and H. Grüner, *Ber.*, **66**, 1945 (1933); **67**, 1582 (1934).

(8) F. Valentin, *Collection Czechoslov. Chem. Communs.*, **8**, 35 (1936).

by its behavior with lead tetraacetate; and by the behavior of its monobenzylidene compound with the same reagent.



II



D-Mannitol

D-Glucitol

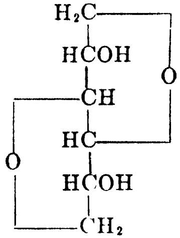
Brigl and Grüner[7] described a third product from their reaction. This was a dibenzoyldianhydro-D-mannitol which on debenzoylation (23) yielded a crystalline product (Brigl and Grüner had described this as a sirup) that was identical with an "isomannide" prepared in 1884 by Fauconnier.[9] The work of Brigl and Grüner[7] on the reciprocal interconversion of isomannide and 1,4-anhydro-D-mannitol, established the presence of the 1,4-anhydro ring in the former. Isomannide was not oxidizable by lead tetraacetate. Its extremely slow reaction with triphenylmethyl chloride was considered to demonstrate the absence of primary hydroxyl groups, leaving only the 1,4:2,6 (1,5:3,6) or 1,4:3,6 structures as possibilities. Synthetic experiments[10] eliminated the former and thus the structure of isomannide was established as 1,4:3,6-dianhydro-D-mannitol. The same conclusion was attained independently through other methods by Wiggins.[11] Entirely analogous arguments

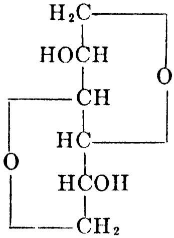
(9) A. Fauconnier, *Bull. soc. chim.*, [2] **41,** 119 (1884).

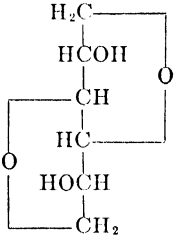
(10) R. C. Hockett and Elizabeth L. Sheffield, *J. Am. Chem. Soc.*, **68,** 937 (1946).

(11) L. F. Wiggins, *J. Chem. Soc.*, 4 (1945).

were deduced (22) for the assignment of the 1,4:3,6-dianhydro structure to a crystalline "isosorbide." In addition the very slow rate of replacement by iodine of one *p*-toluenesulfonoxy group in the di-*p*-toluenesulfonate derivative, was offered as evidence for the absence of primary hydroxyl groups. Further pertinent evidence was obtained (17, 25) when it was found that hydrogenation of isosorbide or isomannide at 200° over nickel at 250 atmospheres pressure yielded a dianhydrohexitol identical with that (isoidide) obtained by the acid dehydration of L-iditol. Apparently dehydrogenation with subsequent reduction and epimerization on carbons 2 and 5 of D-mannitol and on carbon 5 of D-glucitol had taken place. Thus carbons 2 and 5 are open in these three derivatives, confirming the 1,4:3,6 dianhydro structure.


1,4:3,6-Dianhydro-
D-glucitol
(isosorbide)


1,4:3,6-Dianhydro-
D-mannitol
(isomannide)


1,4:3,6-Dianhydro-
L-iditol
(isoidide)

A commercial synthesis of D-mannitol was originally desired by the Atlas Powder Company because its hexanitrate is a useful explosive in blasting caps and explosive rivets. The process employed was that described in the Creighton patents[2] and consisted in the electroreduction of D-glucose under rather strongly alkaline conditions. After the removal of the D-mannitol by crystallization, there remained a sirup or "molasses" whose composition was of interest. From this product the following substances were isolated (20, 26) in crystalline condition or in the form of crystalline derivatives: sorbitol, D-mannitol, allitol, 1-desoxy-D-mannitol (D-rhamnitol), 2-desoxy-D-glucitol and a 2-desoxyhexitol of undetermined configuration. From a product manufactured under milder conditions of alkalinity there was isolated (19): sorbitol, D-mannitol, and 2-desoxy-D-glucitol. These isolations were effected by a combination of crystallization, distillation and extraction techniques in which the formals were found to be useful intermediates. Improved methods for the introduction and removal of the methylene groups were found. There was later devised another technique of separation which is widely applicable for the separation of sugars and their derivatives. This is the chromatographic separation upon clay employing a mixture of an alcohol and water as the developing agent (27). While this method was not employed in the published scientific studies of these complex sugar alcohol mixtures, it is being presently employed as an analytical method in the laboratories of the Atlas Powder Company.

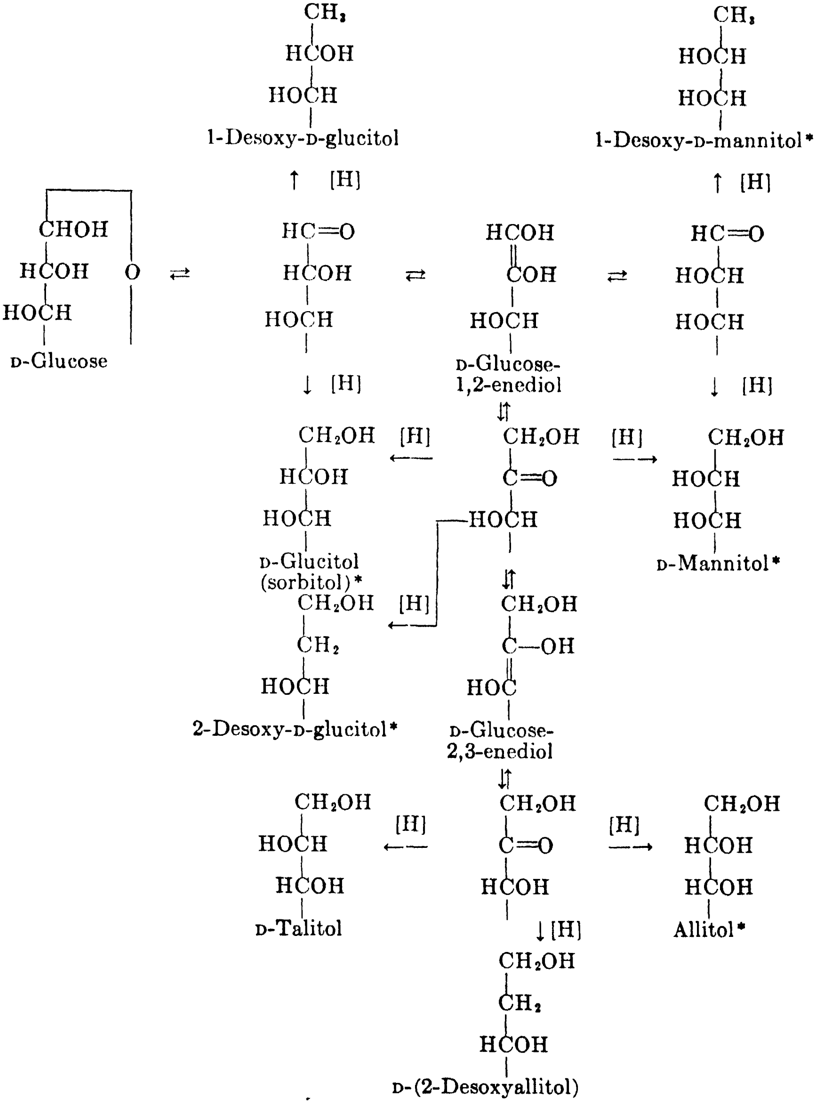


FIG. 1.—Interconversion of D-glucose under reducing conditions. Substances marked with an asterisk are those isolated and identified.

The nature of the isolated substances enumerated above is readily explained on the basis of sugar interconversion through the 1,2 and 2,3-enediols[12] with subsequent reduction of the interconversion products. The compounds predicted and in part isolated are shown in Fig. 1. The reduction of the carbonyl group to the hydrocarbon stage was noteworthy and unexpected. An analytical investigation (16) employing methylation and reduction techniques and carried out on a product obtained by the alkaline electroreduction of D-glucose under rather vigorous conditions, demonstrated the presence of branched chain sugar alcohols. Here it would appear that intermediates produced by carbon skeleton rearrangements of the benzilic acid type led to reduced products corresponding in their carbon nucleus to the saccharinic acids obtainable from the reducing sugars by the action of alkali under non-reducing conditions. That some chain degradation occurred also was indicated by the detection of small amounts of branched chain pentitols (16, 26).

A crystalline product isolated from a manufacturing plant run of an alkaline electroreduction of D-glucose was found to be D,L-glucitol (28). This racemate crystallized so much more readily than either of its components that it was isolable from a large excess of D-glucitol. It had not been previously described. The production of L-glucitol is a notable transformation from the D- to the L-series. Its formation is best explained by the assumption of a dehydrogenation at the cathode surface to form a new carbonyl group with subsequent reduction to the carbinol stage.

M. L. Wolfrom

SCIENTIFIC PUBLICATIONS OF R. MAX GOEPP, JR.

(13) WITH F. D. CHATTAWAY, "The Condensation of Formaldehyde with *p*-Nitrophenol," *J. Chem. Soc.*, 699 (1933).

(14) WITH K. R. BROWN, "Resins from Mannitol and Sorbitol," *Ind. Eng. Chem.*, **30**, 1222 (1938).

(15) WITH ROBERT S. ROSE, JR., "Rotations of *d*-Sorbitol and *d*-Mannitol; the Polymorphism of *d*-Sorbitol," Abstracts of Papers, 97th Meeting, Am. Chem. Soc., Baltimore, Maryland, April 3–7, p. 1R (1939).

(16) WITH S. SOLTZBERG, "Non-hexitol Byproducts from the Alkaline Electroreduction of Hexoses," Abstracts of Papers, 99th Meeting, Am. Chem. Soc., Cincinnati, Ohio, April 8–12, p. 2R (1940).

(17) WITH H. G. FLETCHER, JR., "1,4;3,6-Hexitol Dianhydride, L-Isoidide," *J. Am. Chem. Soc.*, **67**, 1042 (1945).

(18) WITH S. L. HUMOLLER, M. L. WOLFROM AND B. W. LEW, "L-Talitol," *J. Am. Chem. Soc.*, **67**, 1226 (1945).

(19) WITH M. L. WOLFROM, M. KONIGSBERG AND F. B. MOODY, "Sugar Interconversion under Reducing Conditions. I," *J. Am. Chem. Soc.*, **68**, 122 (1946).

[12] See W. L. Evans, *Chem. Revs.*, **31**, 537 (1942).

(20) WITH M. L. WOLFROM, F. B. MOODY AND M. KONIGSBERG, "Sugar Interconversion under Reducing Conditions. II," *J. Am. Chem. Soc.*, **68**, 578 (1946).

(21) WITH S. SOLTZBERG AND W. FREUDENBERG, "Hexitol Anhydrides. Synthesis and Structure of Arlitan, the 1,4-Monoanhydride of Sorbitol," *J. Am. Chem. Soc.*, **68**, 919 (1946).

(22) WITH R. C. HOCKETT, H. G. FLETCHER, JR., AND ELIZABETH L. SHEFFIELD, "Hexitol Anhydrides. The Structure of Isosorbide, a Crystalline Dianhydrosorbitol," *J. Am. Chem. Soc.*, **68**, 927 (1946).

(23) WITH R. C. HOCKETT, H. G. FLETCHER, JR., ELIZABETH L. SHEFFIELD AND S. SOLTZBERG, "Hexitol Anhydrides. The Structures of the Anhydromannitols of Brigl and Grüner. The Structure of Isomannide," *J. Am. Chem. Soc.*, **68**, 930 (1946).

(24) WITH R. C. HOCKETT AND M. ZIEF, "Hexitol Anhydrides. The Structure of the '2,5-Anhydromannitol' of Brigl and Grüner (2,5-Anhydrosorbitol)," *J. Am. Chem. Soc.*, **68**, 935 (1946).

(25) WITH H. G. FLETCHER, JR., "Hexitol Anhydrides. 1,4,3,6-Dianhydro-L-iditol and the Structure of Isomannide and Isosorbide," *J. Am. Chem. Soc.*, **68**, 939 (1946).

(26) WITH M. L. WOLFROM AND B. W. LEW, "Sugar Interconvension under Reducing Conditions. III," *J Am. Chem. Soc.*, **68**, 1443 (1946).

(27) WITH M. L. WOLFROM AND B. W. LEW, "Chromatography of Sugars and Related Polyhydroxy Compounds," *J. Am. Chem. Soc.*, **67**, 1865 (1945); **68**, 1449 (1946).

(28) WITH M L. WOLFROM, B. W. LEW AND R. A. HALES, "Sugar Interconversion under Reducing Conditions. IV. D,L-Glucitol," *J. Am. Chem. Soc.*, **68**, 2342 (1946).

(29) WITH W. W. PIGMAN. Book, "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y. (1948).

(29*a*) WITH R. LOHMAR, "The Hexitols and Some of their Derivatives," *Advances in Carbohydrate Chem.*, **4**, *in press* (1948).

PATENTS

(All assigned to the Atlas Powder Company, Wilmington, Delaware)

(30) "Cast Synthetic Resinous Products," U. S. Pat. 2,111,622 (1938).

(31) WITH J. T. POWER, "Golf Balls," Brit. Pat 483,977 (1938).

(32) "Resinous Reaction Products of Inner Ethers and Natural Resin Acids," U. S. Pat. 2,134,430 (1939).

(33) "Crystallization of Sorbitol," U. S. Pat. 2,315,699 (1943).

(34) "Tobacco Conditioning Agent," U. S. Pat. 2,371,389 (1945).

(35) "Plasticized Vinyl Resins," U. S. Pat. 2,387,841 (1945).

(36) "Cellulose Esters Compositions," U. S. Pat. 2,394,439 (1946).

HISTORICAL ASPECTS OF EMIL FISCHER'S FUNDAMENTAL CONVENTIONS FOR WRITING STEREO-FORMULAS IN A PLANE

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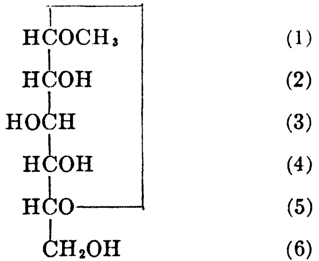
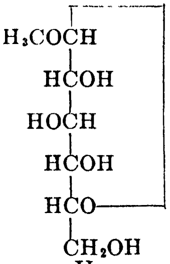
I. INTRODUCTION

It has become general practice to write the stereo-formulas for the anomeric[1] forms of methyl D-glucopyranoside as I and II, and also to employ the alternative formulas Ia and IIa or Ib and IIb. Through established conventions and definitions these stereo-formulas that are written in a plane, and independently also the assigned systematic



I
Methyl α-D-glucopyranoside
([α]D + 159°)



II
Methyl β-D-glucopyranoside
([α]D − 34°)

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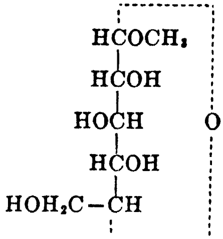
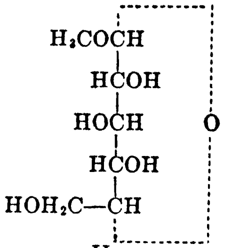
[1] C. N. Riiber and N. A. Sørensen (*Kgl. Norske Videnskab. Selskabs, Skrifter*, No. 7 (1933)) introduced the term "anomer" as a class name in the carbohydrate group for such substances as the α- and β-forms of reducing sugars and their glycosides; thus the two known crystalline forms of D-glucose are the cyclic α- and β-D-glucopyranose anomers, differing by the order of arrangement of the attachments to carbon atom 1.

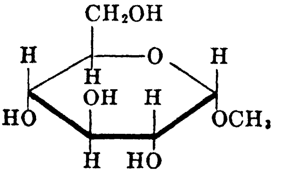


Ia
Methyl α-D-glucopyranoside

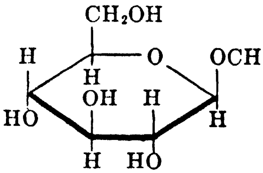
(1)
(2)
(3)
(4)
(5)



IIa
Methyl β-D-glucopyranoside



Ib
Methyl α-D-glucopyranoside



IIb
Methyl β-D-glucopyranoside

names, define the mechanical models of the two substances in three-dimensional space. It is the purpose of the present article to review the historical origins of these unequivocal stereo-formulas and names in order that the application of the conventions and definitions that underlie them, to the structures of carbohydrates in general, may have full clarity. Since formulas Ia, Ib, IIa and IIb were introduced at a much later date and are derived from the earlier ones, attention will be given first to the historical origin of formulas I and II.

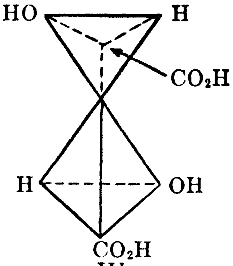
II. Fischer's Projections of the Stereo-models

The stereo-formulas I and II represent three-dimensional objects projected upon the plane of the paper in a highly conventionalized manner. The three-dimensional objects are the mechanical models of molecules that are universally accepted as showing the gross order of arrangement in space of the component atoms, as derived from the application of the principles of the Van't Hoff-Le Bel theory of the asymmetric carbon atom to a large collection of experimental facts. The inferred gross space relationships that are shown by these models, namely, the attachments of atom to atom by primary chemical bonds, are independent of all nomenclature, as was emphasized by Fischer[2]; it is to these models themselves that one should give attention in any decisions that concern fundamental matters, including the devising of artificial systems of nomenclature and of rules of rotation and reactivity.

---

[2] E. Fischer, *Ber.*, **40**, 102 (1907). ["Untersuchungen über Kohlenhydrate und Fermente," Verlag Julius Springer, Berlin, vol. **1**, p. 893 (1909).]

If one seeks to illustrate on paper or on the blackboard or screen of a lecture room a particular mechanical model, he does so by using some kind of plane projection of it, its flat "picture." The kind of flat "picture" that is understood by all without any statement of conventions for projection is the photograph, but for obvious reasons photographs of actual models are not suitable for extensive use in ordinary written or printed records; instead, one uses for convenience outline drawings simulating the photograph. Even this simplification is not sufficient for the most economical printing of chemical writings because the making of the required cuts can be expensive. Such practical considerations often influence the selection of the printed formulas of stereochemistry. Van't Hoff sometimes used in his writings[2a] on stereochemistry line drawings representing tetrahedra in picture form. Such a picture for the model of mesotartaric acid could be III. One sees that the order of the apices for the lower tetrahedron, *when viewed from the central apex that unites the two tetrahedra,* is clockwise for H → OH; when the upper tetrahedron is viewed *from this same central apex* the order H → OH is counterclockwise. This opposition of orders persists no matter how the tetrahedra are turned, and the compensation which results makes the model a *meso* structure representing the unresolvable form of optically



III

(2a) Original copies of J. H. Van't Hoff's very early brochures on stereochemistry are now rare. The first brochure, in Dutch, was published at Utrecht in 1874; an English translation of it may be found in G. M. Richardson's "The Foundations of Stereo Chemistry," Scientific Memoirs Series, American Book Company, New York (1901) (copy in the Library of Congress, Washington, D.C.), and its translation of the Dutch title is "A suggestion looking to the extension into space of the structural formulas at present used in chemistry, and a note upon the relation between the optical activity and the chemical constitution of organic compounds." That library possesses originals of the second and third brochures, in French, with the titles "La chimie dans l'espace" (1875) and "Dix années dans l'histoire d'une theorie" (1887). J. A. Le Bel's first article is readily accessible in the *Bull. soc. chim.*, [2] **22,** 337 (1874). Van't Hoff's age was twenty-two and Le Bel's twenty-seven when they published almost simultaneously but entirely independently the concept of an asymmetric carbon atom.

inactive tartaric acid. Van't Hoff invented a conventionalized way for specifying such a model by symbols that are easy to print; he designated one tetrahedron as + and the other as −, and thus the sum for them becomes zero in suggestion of the *meso* character of the acid. One of the active tartaric acids can be designated ++ by the same plan and its enantiomorph becomes − −. This shorthand method of specifying the mechanical model serves the purpose when one is considering the space relations of these rather simple types of substances, containing only two asymmetric carbon atoms. The method leads to troublesome pitfalls, however, when one attempts to apply it to all the members of a more complicated group of substances containing more than two asymmetric carbon atoms in their molecules, as for example the pentose, hexose and higher-carbon sugars and the corresponding alcohols and acids. Previous to the time of Fischer's proof[3] of the configuration of glucose and some of its relatives, Van't Hoff had predicted from the theory of the tetrahedral asymmetric carbon atom that the number of possible aldohexoses of normal unbranched structure is sixteen, representing eight pairs of enantiomorphs, and he classified these hypothetical structures by his + and − notations; his table is here reproduced as printed in Fischer's article.[3a]

TABLE I

Van't Hoff's Table of Signs for the Sixteen Possible Aldohexoses and the Corresponding Alcohols and Dibasic Acids

+	+	−	−
+	−	+	−
+	−	+	−
+	+	−	−
1	2	3	4

11	12	13	14	15	16
+	+	+	+	+	−
+	+	+	−	−	+
+	−	−	+	−	−
−	+	−	−	−	−
−	+	−	−	−	−
+	−	−	+	−	−
+	+	+	−	−	+
+	+	+	+	+	−
5	6	7	8	9	10

He also predicted from this geometric theory that the reduction of these sixteen aldohexoses would lead to only ten hexitols, of which two would be *meso* structures [7 = 13 (allitol) and 8 = 14 (dulcitol)],[4] and the

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(3) (*a*) E. Fischer, *Ber.*, **24,** 1836 (1891); (*b*) *ibid.*, **24,** 2683 (1891). ["Untersuchungen," pp. 417, 427.]

(4) The indicated identifications were of course later experimental discoveries, mostly by E. Fischer.

remaining eight would represent four pairs of enantiomorphs [1 and 4 (mannitols); 2 and 3 (iditols); 5 = 11 and 9 = 15 (sorbitols); 6 = 12 and 10 = 16 (talitols)]. This reduction in number and the production of *meso* structures result because the end carbon groups of the hexitols are alike. The same considerations naturally apply to the corresponding dibasic acids. Let the reader examine the notations of structures 7 and 13 in the table, *remembering that the orders for the H and OH attachments are read by viewing them from the center of the structure.* As applied to dibasic acids, 7 and 13 represent one and the same acid of *meso* configuration. A similar result comes from the structures 8 and 14, which thus represent a single second *meso* dibasic acid. Fischer[5] showed that mucic acid must be limited to one of these *meso* configurations, but a decision between them was not possible at that time. The + and − notations of this table are very confusing today because, as should be stated again for emphasis, *the signs are given by observing the tetrahedra of the mechanical model from a point within the model, namely, its center.* It will be recalled that this convention was used by Van't Hoff for classifying the various tartaric acids; he extended the convention to the more complicated substances of the table, and it is in the consideration of them that one meets the following pitfall, as was described by Fischer in a supplementary article[3b] sent in two months after dispatching his first article[3a] on the configuration of glucose and its isomers. The matter will be explained here in some detail, using quotations (in translation) from the second article, because the subject is treated so cursorily in most college textbooks, even when mentioned at all, that few students today understand the historical background and the origin of our present conventions for writing the stereo-formulas of general organic chemistry. In the second article Fischer analyzes the + and − notation of Van't Hoff's table and shows why it should be discarded in the interest of clarity.

> In the first article I have developed for grape sugar the formula
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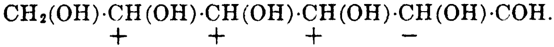
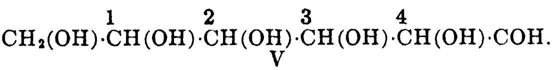
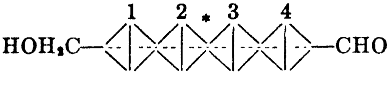
> 
>
> $$CH_2(OH){\cdot}\underset{+}{CH(OH)}{\cdot}\underset{+}{CH(OH)}{\cdot}\underset{+}{CH(OH)}{\cdot}\underset{-}{CH(OH)}{\cdot}COH.$$
>
> IV
>
> However, the designation of arrangement in space as + or −, which was introduced by Van't Hoff and retained unchanged by me, can lead easily in case of such complicated molecules to an erroneous interpretation. In order to avoid this I regard it as necessary that a more detailed interpretation of the formula be stated, and for this purpose I begin by designating the four asymmetric carbon atoms through the use of the numbers 1 to 4.
>
> 
>
> $$CH_2(OH){\cdot}\overset{1}{CH(OH)}{\cdot}\overset{2}{CH(OH)}{\cdot}\overset{3}{CH(OH)}{\cdot}\overset{4}{CH(OH)}{\cdot}COH.$$
>
> V

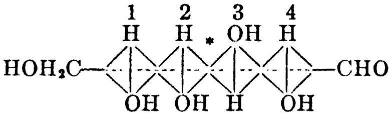
(5) E. Fischer and J. Hertz, *Ber.*, **25**, 1247 (1892). ["Untersuchungen," p. 459.]

> In the general considerations by Van't Hoff, which underlie my special deductions, carbon 1 is compared only with 4, and likewise 2 only with 3. Thus in grape sugar the order of the hydrogen and hydroxyl attachments to carbon atom 1 is the reverse of that for carbon atom 4; on the other hand, the orders for carbon atoms 2 and 3 are alike. It is possible also to compare carbon atom 1 with the two middle carbon atoms; this has been done by bringing xylose and arabinose into relationship with grape sugar, with the result that the order of arrangement of hydrogen and hydroxyl on carbon atom 1 comes out to be the same as for carbon atom 3. On superficial consideration it might seem that this result shows that carbon atoms 1 and 2 have the same order; in reality, however, just the opposite conclusion is the true one. Through the use of a model it is readily recognized that in the case of carbon atom 2 its sign changes according as it is compared with carbon atom 1 or with 3.

Fischer did not amplify the last sentence of this quotation but left the matter for the interested reader to work out "readily" for himself through a close study of the mechanical model. The writer believes that in general there is a real need for instructors to explain, and for students to comprehend clearly, the details of the reasons which led Fischer to discard the + and − notation of Van't Hoff and to introduce the system for writing configurational formulas that is now in universal use. Let the inquiring student first convince himself of the correctness of Fischer's quoted remarks and of the original formula IV of the Van't Hoff notation by inspecting actual tetrahedra laid on a table as shown in figures VI and VII. The plus and minus signs of formula IV for grape sugar signify that when each of the four tetrahedra is viewed from the apex (*) joining 2 and 3 the order of the H and OH attachments to each is the same for tetrahedra 1, 2 and 3 but is in the opposite direction for tetrahedron 4. If these attachments are placed at the free apices of the four tetrahedra there results the conventional model VII for natural grape sugar provided that the plus sign is selected to mean counterclockwise order for H → OH. If one selects the plus sign to mean clockwise order for H → OH, the customary model for the enantiomorph of natural grape sugar results. Notice that the signs in formula IV are conditioned by the selected point of observation, namely the apex joining 2 and 3. If the point of observation be the apex between 1 and 2 the signs become + − + −. In Van't Hoff's system the relative orders for any two tetrahedra are obtained by viewing them from a point between the two tetrahedra; Fischer called attention to the fact that if this system be used in designating such a configuration as IV, the sign of carbon atom 2 changes



VI

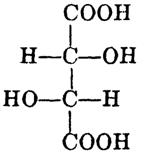


VII

according as this atom is compared with carbon atom 1 or with 3. It is as though an observer might read a transparent clockdial from in front and then go behind the dial and read it again; naturally, he would find that the numbers run clockwise in the first case and counterclockwise in the second one. Formula IV is not ambiguous but it is easily subject to misinterpretation; accordingly, Fischer discarded the confusing + and − system entirely and substituted for it a method of representing the tetrahedral space relationships by plane projections that are simple to write and remember, easy to understand and visualize as representing mechanical models, and relatively inexpensive to print. This method necessarily requires the setting up of conventions; the ones that he chose seem as few in number and as simple in character as is possible. Unfortunately, it is rare to find in the college textbooks of organic chemistry a chapter on stereochemistry which emphasizes these fundamental conventions, and many of the widely used and latest textbooks not only do not mention them but even give erroneous formulas for the active tartaric acids through violation of them.

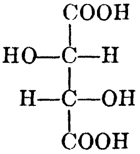
Fischer did not state his *first convention* in a fully generalized form; instead, he indicated the manner in which he proposed to write the formulas for the three tartaric acids and he then wrote the formulas for the substances of the hexose and pentose series in an analogous way. The following is a free translation of his statement.

> First, construct mechanical models for the dextrorotatory, levorotatory and inactive [*meso*] tartaric acids, and then lay these on the plane of the paper in such a way that the four carbon atoms lie in a straight line and the hydrogen and hydroxyl attachments stand above the plane. By projection one obtains then the drawings VIII, IX and X.



$$\begin{array}{c} \text{COOH} \\ | \\ \text{H—C—OH} \\ | \\ \text{HO—C—H} \\ | \\ \text{COOH} \end{array}$$

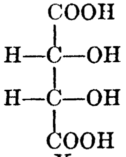
VIII



$$\begin{array}{c} \text{COOH} \\ | \\ \text{HO—C—H} \\ | \\ \text{H—C—OH} \\ | \\ \text{COOH} \end{array}$$

IX

Right and left tartaric acid

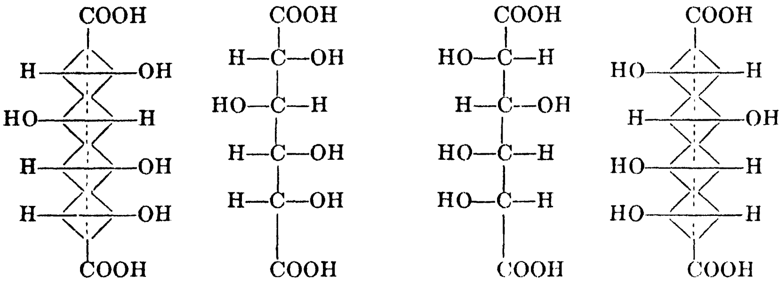


$$\begin{array}{c} \text{COOH} \\ | \\ \text{H—C—OH} \\ | \\ \text{H—C—OH} \\ | \\ \text{COOH} \end{array}$$

X

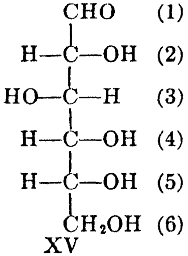
Inactive tartaric acid

Fischer then shows that when the arrangements for the enantiomorphous forms of saccharic acid that are specified by his use in his first article[3a] of Van't Hoff's + and − signs (number 5 = 11 and 9 = 15 of Table I) are given to models, and these are projected as in the case of the tartaric acids, the formulas XII and XIII result. The present author has added the diagrams XI (= XII) and XIV (= XIII) in which the dotted straight line indicates those edges of the tetrahedra that lie in the plane of the paper; this line is dotted to indicate that these edges are not visible to the observer if the tetrahedra are opaque objects. Formulas XII and XIII are sometimes misunderstood through lack of appreciation of Fischer's first convention; formulas XI and XIV include this convention automatically and may therefore be recommended for elementary instruction.


XI XII XIII XIV

Fischer's Formulas (XII and XIII) for the Enantiomorphous Saccharic Acids

Formulas XII and XIII are mirror images representing the two enantiomorphs; Fischer made the arbitrary selection, which may be named his *original second convention*, that formula XII shall be chosen to represent the form of saccharic acid that results from the oxidation of natural glucose. Using these two conventions, he then showed that the configuration of natural glucose (IV) which he specified by the + and − signs in his first article[3a] is to be presented by the equivalent formula XV.


XV

(See also VII)

Fischer's Original Formula for Natural Glucose[6] (modern numbering)

In historical accuracy, it should be remembered that Fischer's original second convention was applied to the enantiomorphous saccharic acids as the reference substances or "standards"; his formula for natural glucose (XV) followed as a sequel. It has now become customary to select the enantiomorphous forms of glyceraldehyde as the reference substances or "standards," from the stereo-formulas of which there follow those of natural glucose, its enantiomorph and the saccharic acids. This change was suggested by Rosanoff as advisable on grounds of logical classification of glucose as a higher-carbon sugar from glyceraldehyde, as will be explained in detail later (page 13). The enantiomorphs of glyceraldehyde were first separated experimentally by Wohl and Momber[7a] and later[7b] these investigators were able to demonstrate that the dextrorotatory glyceraldehyde is the stereo-relative of natural glucose; accordingly, its stereo-formula is written as XVI in order that the arrangements for its asymmetric carbon atom may agree with the prior arrangements for carbon atom 5 in Fischer's stereo-formula for natural glucose (XV). A readily perceived reason for the selection of carbon atom 5 is that through the cyanohydrin synthesis of higher-carbon sugars natural glucose can be regarded as a higher-carbon sugar that belongs in the series of which the dextrorotatory glyceraldehyde is the parent triose, but the fundamental reason is of strictly mathematical geometric nature, as will be explained in section IV.

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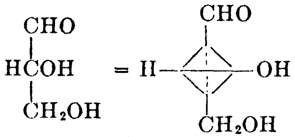
[6] A historical review of the experimental researches by Fischer which led to his establishment of this configurational formula for natural glucose was published by the writer in *J. Chem. Education*, **18**, 353 (1941).



XVI

The Dextrorotatory Glyceraldehyde

III. Fischer's Proposals of Nomenclature in the Carbohydrate Group

The preceding formulas VII and XI to XVI have been written purposely without the use of *d* and *l* symbols for distinguishing the members of a pair of enantiomorphs in order to emphasize that the mechanical models and Fischer's conventional formulas for representing them are quite independent of any plan of nomenclature. Recognizing this independence, let us consider next the historical origin and development of systematic nomenclature for such models and stereo-formulas in the carbohydrate group.

The period during which the foundations of present carbohydrate nomenclature assumed a systematic form covers the years 1890–1895; the system is due to Emil Fischer and its conventions were devised by him because they were necessary for clarity in the description of his sugar researches. His main proposals for nomenclature were made in successive steps during this period, and a study of the order of the historical record leaves no doubt that the subject assumed form in his mind by

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(7) (*a*) A. Wohl and F. Momber, *Ber.*, **47**, 3346 (1914); (*b*) *ibid.*, **50**, 455 (1917).

gradual steps as new experimental facts came to light. What appears to be the first step in this historical order of development is shown through the following free translation from his article[8] in which the naming of the enantiomorphous forms of mannose and mannitol and several of their derivatives is discussed.

> It will be advantageous to retain the old names mannose and mannite in designating the new products, but the distinction of enantiomorphs by the terms dextro- and levo-rotatory seems to me inadvisable on the following grounds. The rotation of a derivative is often of the opposite sign from that of the original substance; for example the phenylhydrazone of the dextrorotatory mannose rotates to the left and that of the levorotatory mannose rotates to the right. The enantiomorphous phenylosazones from mannose and some salts of mannonic acid behave in the same way and unquestionably such reversals will be observed frequently as researches in the sugar group are extended. If various compounds which belong exclusively in one series are designated by the prefixes dextro- and levo-rotatory, mistakes through interchange will be unavoidable. Therefore I suggest that all compounds of such a series be designated according to the direction of rotation of the aldehyde (sugar) either with the letter *d* (*dextro*) or *l* (*levo*) or *dl* (inactive), just as the letters *o*, *m* and *p* are used in the case of benzene derivatives.

At this far later time, it is readily apparent that although this proposal of Fischer's served his immediate need of describing the enantiomorphs in the mannose series, it would soon prove faulty in respect to that part of the definition which implied that each aldose was to be named *d* or *l* from its sign of rotation. This definition, in strict and general application, would specify that natural arabinose, which is a strongly dextrorotatory aldose, should be named *d*-arabinose, and the mannonic acid that results from the application of the cyanohydrin synthesis to natural arabinose could be named either *d*-arabinosecarboxylic acid or *l*-mannonic acid according as it is classified to be a derivative of arabinose or of mannose, respectively. In the very early article from which the foregoing quotation is taken, Fischer used no *d* or *l* symbol with the name "arabinosecarboxylic acid" (the name which Kiliani, the discoverer of the acid, had given to it) but he used as a synonym the designation *l*-mannonic acid through his discovery that the substance is the enantiomorph of the mannonic acid that results from the oxidation of natural mannose, which he named *d*-mannose because it is dextrorotatory. He had synthesized natural levulose from *d*-mannose and accordingly had designated it *d*-levulose.

In the same volume of 1890 there appears an article by Fischer[9] which was received within two months after the one that carries the proposal which has been discussed. He stated that since the use of the words

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[8] E. Fischer, *Ber.*, **23**, 371 (1890, February 8). ["Untersuchungen," p. 331.]

[9] E. Fischer, *Ber.*, **23**, 930 (1890, March 24). ["Untersuchungen," p. 322.]

dextro and *levo* for enantiomorphs, or the letters *d* and *l* which he had suggested, is scarcely avoidable, it was unfortunate that the names dextrose and levulose, which were then current in both scientific and common use, had become customary. Accordingly, he proposed that the name "glucose," which had been suggested years before by Dumas[10] for grape sugar but was then in current use as a class name for the six-carbon sugars or even for reducing sugars in general, be selected to take the place of dextrose and be limited to that sugar, and that a new name "fructose" (the fruit-sugar, from the Latin *fructus*) be given to levulose. His proposal has been accepted generally in scientific writings; in ordinary parlance the names dextrose and levulose have continued in use in some languages, including English.

In June of 1890 in the first of Fischer's[11] three summarizing articles on syntheses in the sugar group, he discusses nomenclature of sugars extensively and makes many proposals for systematization, most of which became general usage in later years. The names glucose and fructose are to replace dextrose and levulose, respectively, in scientific language because the latter terms are wholly unsuitable for meeting the new need of distinguishing enantiomorphs. Natural mannose is to be designated *d*-mannose because it is dextrorotatory; its reduction product is to be named *d*-mannite and its monobasic acid *d*-mannonic acid; these substances belong in the *d*-series. Natural glucose also belongs in this *d*-series because he had synthesized it by the following reactions: *d*-mannonic acid → *d*-gluconic acid (lactone) → *d*-glucose. This designation of natural glucose happened to agree with its dextrorotation. Other synthetic reactions had resulted in the production of natural fructose from natural mannose (or natural glucose) and accordingly he had designated the natural levorotatory ketose *d*-fructose. Starting from natural arabinose, he had found that the hexoses which result from it by his cyanohydrin synthesis of higher-carbon sugars[11a] were the enantiomorphs of natural mannose and natural glucose, respectively, and were to be named accordingly *l*-mannose and *l*-glucose. All these scientific designations remain unchanged today, and the like is true for the class names aldose, ketose, triose, tetrose, pentose, hexose, heptose, octose and nonose, which he suggested at that time. In order that this use of the word triose might not be in conflict with Scheibler's[12] prior suggestion that disaccharides, such as sucrose and lactose, be designated bioses and trisaccharides be designated trioses, Fischer recommended the new terms

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(10) J. B. Dumas, *Compt. rend.*, **7**, 106 (1838).

(11) E. Fischer, *Ber.*, **23**, 2114 (1890). ["Untersuchungen," p. 1.]

(11*a*) See *Advances in Carbohydrate Chem.*, **1**, 1 (1945).

(12) C. Scheibler, *Ber.*, **18**, 646 (1885).

hexobiose, hexotriose and so forth for classes of compound sugars. It is of much interest at present, on account of various recent proposals for precise nomenclature in the group of heptoses and higher-carbon sugars, that Fischer made the following recommendation, which is here quoted in translation.[11]

"The sugars will be named according to the number of carbon atoms as triose, tetrose, pentose, hexose, heptose, octose and nonose and particular isomers will be distinguished by the use of a syllable which indicates the source."

Since common names had proved so satisfactory for the hexoses and lower-carbon sugars, he applied his suggestion only to heptoses and their higher-carbon sugars; the higher-carbon sugars from glucose that were then known thus received the names glucoheptose and glucooctose.[13] Fischer also introduced the names galaheptose and galaoctose and similar ones in the mannose series.

Fischer proposed the class name methylpentose for rhamnose and its relatives. It is of some interest that he used on one occasion[11] the word "ose" as a class name in the sugar group.

IV. ROSANOFF'S MODIFICATION OF FISCHER'S PROPOSALS OF NOMENCLATURE

In subsequent years it became apparent that Fischer's plan for naming enantiomorphous forms of sugars and their derivatives by the *d* and *l* symbols is not as simple, precise and logical as is possible. For example, Fischer had come to use the designations *l*-arabinose and *l*-xylose for the naturally occurring forms of these pentoses, both of which are dextrorotatory; these symbols constituted a tacit reversal of his early proposal[11] that aldoses be designated *d* or *l* according to their rotations. The first chemist to publish a critical review of the matter was M. A. Rosanoff,[14] who was at that time a young instructor in New York University. He emphasized the following points. (1) The *d* and *l* symbols for configurations are not different from those that were in current use for rotations;

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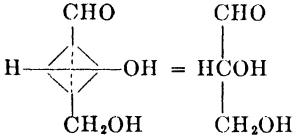
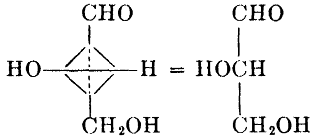
(13) It seems to the writer that the names D-gluco-D-*gulo*-heptose and D-gluco-D-*ido*-heptose which he has suggested [C. S. Hudson, *J. Am. Chem. Soc.*, **60**, 1537 (1938)] for the two glucoheptoses that Fischer synthesized and named α- and β-glucoheptose, are preferable to the designations D-*glycero*-D-*gulo*-heptose and D-*glycero*-D-*ido*-heptose which some chemists have substituted. The expansion of the old name D-glucoheptose by the introduction of the syllable D-*gulo* or D-*ido* to indicate the full configuration conforms to the general rules of organic nomenclature and is not the violation of them that has been inferred erroneously in some quarters. In writing these names the *gulo* and *ido* syllables should be italicized, but not the gluco syllable, which is a part of the original name glucoheptose.

(14) M. A. Rosanoff, *J. Am. Chem. Soc.*, **28**, 114 (1906).

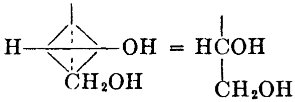
this was a matter of minor importance which could be remedied by using other symbols and Rosanoff suggested the Greek letters δ and λ for configurations. (2) The naming of natural xylose as *l*-xylose solely because *l*-saccharic acid had been synthesized from it followed a plan in which the criterion for *l* or *d* involves the particular synthetic correlation that happened to be first in historical order. This second criticism affected fundamentals. Rosanoff advocated the establishment of a system for δ and λ classifications that would be based upon space arrangements of the groups in the mechanical molecular models of the substances and would involve only mathematical geometric conventions. Such a geometric system of classification could be based upon the most simple of all possible selections, namely, the selection of one of the mirror-image formulas XVII and XVIII (representing the theoretically possible active forms


XVII

and


XVIII

of glyceraldehyde, which had not then been isolated) as the space formula for this particular terminal asymmetric carbon atom (XIX) in the models of all sugars that are placed in the δ category. In order to make this selection conform with Fischer's second convention by which formula VII (= XV) had already been assigned to δ-glucose by Fischer, Rosanoff


XIX

proposed that the configuration XVII of one of the hypothetical glyceraldehyde enantiomorphs be given in advance the designation δ-glyceraldehyde and that XVII be selected as the reference formula for all the sugars of the δ-series. (The preparation and resolution of the enantiomorphs of glyceraldehyde and the assignment of Rosanoff's formula XVII to the dextrorotatory glyceraldehyde by Wohl and Momber[7] eleven years later has been referred to on p. 9.) In making such a fundamental convention it is not essential to introduce the experimental facts of the cyanohydrin synthesis; the space relationships that are expressed by the various models are abstract geometric ones, and the classification of these models as geometric structures is wholly an exercise in pure mathematics. The ancient geometers, who first used the name tetrahedron and even distinguished between regular and irregular tetrahedra, could have constructed all these sugar models and classified them into two enantiomorphous δ and λ families of mechanical models, purely as a mathematical exercise. The place of importance that is occupied by the experimental facts which have been discovered in later times through the cyanohydrin and other reactions is in the correlation of many of these geometric models with *individual substances* of the sugar group. To classify these geometric models by a criterion that can vary with the experimental method through which the correlation happened to be discovered in each individual case cannot be expected to result in an orderly and logical system. The changes that an acceptance of Rosanoff's suggestions would have required at that time were limited to a reversal of the older *d* and *l* prefixes of Fischer's for xylose, gulose, idose, sorbose, and threose. It was fortunate that so small a number of sugars required a change of *d*-, *l*-classification through Rosanoff's proposals. Fischer had named natural galactose *d because it is dextrorotatory*[15] just as he had named natural mannose *d* for the same reason; by lucky chance this symbol for natural galactose and the related talose fitted the Rosanoff systematic classification. Fischer was not in favor of Rosanoff's proposals; the details[15a] of his objections to them may be passed over because they did not prevent the present universal acceptance of what may be designated appropriately *the Fischer-Rosanoff system of naming the two enantiomorphous series in the carbohydrate group.* In this system the *d* configurational symbol, which many writers have preferred for some years past to print in small capital Roman type,[16] D, applies to a specified glyceraldehyde enantiomorph as the reference substance and it is designated D-glyceraldehyde, the symbol being spoken as "dee." It is purely coincidental that this enantiomorph happens to be the dextrorotatory one and therefore D is never to be regarded as implying rotation. Natural glucose has been correlated experimentally with this D-glyceraldehyde by Wohl and Momber[7b] and is therefore designated D-glucose

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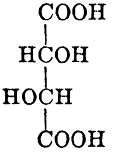
(15) E. Fischer and J. Hertz, *Ber.*, **25**, 1247 (1892); E. Fischer and R. S. Morrell, *Ber.*, **27**, 382 (1894). ["Untersuchungen," pp. 460, 504.]

(15*a*) E. Fischer, *Ber.*, **40**, 102 (1907). ["Untersuchungen," p. 895.]

(16) Private information from Dr. Rosanoff has recently brought to light an interesting historical matter. In the manuscript of his original article and on the figure which accompanies it he used the capital D and L. The editor of the journal persuaded him to recommend instead the Greek δ and λ, and the manuscript was changed accordingly. The figure, however, was not altered and thus the reader finds the original D and L in it. Rosanoff's proposal of the δ and λ symbols has not been accepted by others. The present writer was first impressed with the advantage of printing the symbols as the small capital Roman D and L through correspondence with Dr. Austin M. Patterson, who had used them in his table of organic compounds in the twentieth edition of the "Handbook of Chemistry and Physics" [Chemical Rubber Publishing Co., Cleveland, Ohio (1935)].

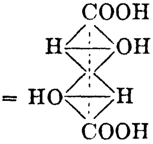
and assigned the formula XV in which the hydroxyl group on carbon atom 5 is to the right. (Modern usage begins the numbering of the sugar from the reducing end.)

Ordinary (that is, dextrorotatory) tartaric acid (XX) corresponds to XXI and not to XXII, as is often stated erroneously in textbooks. Formula XXII applies to the enantiomorph of natural tartaric acid. Formulas XXI and XXIII are drawn in conformity with Fischer's first convention but formula XXII does not follow it.

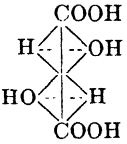


$$\begin{array}{c} COOH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ COOH \end{array}$$

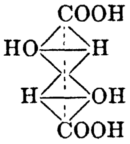
XX
L-Tartaric Acid
(dextrorotatory)



XXI



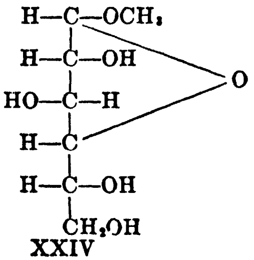
XXII



XXIII
D-Tartaric Acid
(levorotatory)

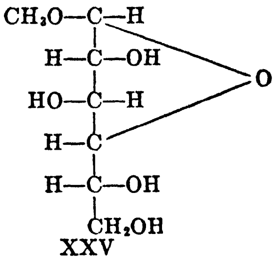
V. The Classification of Anomers by the Symbols α-D, α-L, β-D and β-L

Two years after Fischer's rejection of Rosanoff's suggestions the writer published an article on "the significance of certain numerical relations in the sugar group."[17] The existence of two methyl D-glucosides which Fischer discovered in his early researches was attributed by him to the asymmetry which would be developed at carbon atom 1 if a hemiacetal ring should be present in the glucosides. Assuming such a ring to connect carbon atoms 1 and 4, by analogy with lactone formation from hydroxy acids, he wrote[18] the formulas for the glucosides as XXIV and XXV; it was not possible of course to distinguish which formula applied to a particular glucoside. Fischer named the first of these glucosides to be discovered "α-methyl-*d*-glucoside" ([α]D = +159°) and its anomer "β-methyl-*d*-glucoside" (−34°). He also discovered the



XXIV

and



XXV

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[17] C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

[18] E. Fischer, "Untersuchungen," p. 88.

enantiomorphs of this pair of glucosides, and he[19] named them "α-methyl-*l*-glucoside" (−159°) and "β-methyl-*l*-glucoside" (+34°). The attention of the reader is especially directed to these names. *It should be carefully noted that the two members of each pair of enantiomorphs bear customary opposite symbols (corresponding to the present* D *and* L*) for the parent sugar but one symbol only (α for one pair, β for the other) in designation of the asymmetric arrangement on carbon atom 1. It is obvious therefore that the α-*D*- and β-*L*-glucosides possess an identical order of arrangement of the groups that are attached to carbon atom 1, and that the β-*D*- and α-*L*-glucosides possess in common the opposite arrangement.* It is to be noticed that these symbols are composite ones; an α symbol without the D or L is ambiguous because α-D- and α-L- designate opposite orders of arrangement of the groups on the anomeric carbon atom. When Fischer discovered the two similar anomers from natural D-xylose, a sugar which he had named *l*-xylose, he[19] designated the dextrorotatory form "α-methylxyloside" (+153°) and the levorotatory isomer "β-methylxyloside" (−66°) and expressed the view that the arrangement of groups on carbon atom 1 is probably the same in the α-form of his "*l*-xyloside" and the α-form of the D-glucoside because of the close similarity of the configurations for D-glucose and natural xylose (his "*l*-xylose,") and the similarity of the rotations of their methyl glycosides. When the writer found that there are certain additive numerical relations among the rotations of the glycosides of many of the sugars, which can be interpreted to indicate relative configurations of anomers by the hypothesis of optical superposition, the presentation of the new subject required close attention to systematic nomenclature. The following of Fischer's practice of naming natural xylose an *l* sugar led to the contradiction that in the D- and L-glucose series α-D- and β-L- meant one arrangement (+A) and β-D- and α-L- meant the opposite arrangement (−A), but that in the xylose series these meanings would become reversed and α-L- would designate the +A rather than the −A arrangement. If the suggestions of Rosanoff were adopted, such confusing contradictions would disappear and an orderly naming of anomers would be possible; accordingly, the writer chose the Rosanoff modification of the Fischer system in order that the development of configurational relationships of anomers in the sugar group might be expressible in clear language through the use of a precise system of nomenclature. At the present time the Fischer-Rosanoff system has received universal acceptance.[20]

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(19) E. Fischer, *Ber.*, **28**, 1145 (1895).

(20) The Fischer-Rosanoff system has been occasionally referred to as the Wohl-Freudenberg system through lack of regard for the clear historical record. A. Wohl and K. Freudenberg [*Ber.*, **56**, 309 (1923)] adopted the Fischer-Rosanoff system years after it had been followed by many other chemists in numerous publications.

The experimental facts from which the writer inferred[17] his isorotation rules indicated that the arrangements of the groups that are attached to carbon atom 1 in the α- and β-forms of sugars and glycosides of supposedly one and the same type of ring structure, as was then assumed, could be brought into a correlated configurational system through a knowledge of the relative values of the rotations of the substances. The difference in molecular rotation between the members of an α,β-pair was so great that a complete inversion of the relationship seemed highly improbable. At that time there was no way known for correlating such configurations except through enzyme actions in a very few instances, or through these new rotatory relations, which were seen to be of wide applicability. Accordingly, the writer set up rules for naming such α and β anomers which may be stated today in the following form.

In the D-*series the more dextrorotatory member of an α,β-pair of anomers is to be named α-*D-, *the other being β-*D-. *In the* L-*series the more levorotatory member of such a pair is given the name α-*L- *and the other β-*L-.

These rules are simple and they are not of a wholly empirical nature, as some commentators have stated. This is evident from the foregoing description of their derivation since they are based on the hypothesis that optical superposition holds for these substances of the sugar group in an approximation that is at least sufficient to exclude a complete reversal of relative rotations for an α,β-pair of anomers. The data indicated at that time that this limited application of the hypothesis is well founded, and all the numerous subsequent pertinent data have confirmed this view. Obviously, these rules are to be regarded as subject in every case to the ultimate establishment of the three-dimensional model for the particular substance; their practical usefulness lies in the fact that they apparently do correlate configuration and rotation on the basis of a theory (optical superposition) which, though not mathematically exact, holds sufficiently closely in the carbohydrate group to justify its use in allocating anomeric configurations. It has always been apparent that precise application of the rules requires a knowledge of the structure of the substance and its proper place in the Fischer-Rosanoff D or L system of nomenclature. When such knowledge is lacking or incomplete, as is the case for some sugars and derivatives even at the present time, the nomenclature rules can be applied only provisionally.

The placing of the methoxy group on the right of carbon atom 1 in formula I and on the left in formula II is not an arbitrary allocation at the present time, but is a conclusion from strong experimental evidence that has been obtained in late years. The details of this evidence are not included in the present review but its nature may be understood from the following illustration. The addition of methyl alcohol to the ethylene oxide ring of the triacetate of D-glucopyranose 1,2-anhydride at ordinary temperature and without a catalyst proceeds rapidly and yields nearly quantitatively methyl β-D-glucopyranoside triacetate. From numerous examples of the behavior of ethylene oxide rings in various types of substances there has resulted the generalization that the ring opens to give a *trans* arrangement; accordingly the formula II is written to indicate that the methoxyl group on carbon atom 1 is in *trans* position to the hydroxyl group on carbon atom 2,[21] an arrangement which is readily visualized from formula IIb.

VI. HAWORTH'S PERSPECTIVE FORMULAS

We pass now to a description of the historical origin of such carbohydrate formulas as are represented on page 2 by Ia, Ib, IIa and IIb. When Haworth[22] and his coworkers discovered that the tetramethyl-D-glucose which results from the acid hydrolysis of either the α- or β-anomer of fully methylated normal methyl glucoside is a 2,3,4,6-tetramethyl-D-glucose, it became apparent that the oxygen bridge connects carbon atoms 1 and 5. In the abnormal or "γ" type of glucoside this bridge was found to connect carbon atoms 1 and 4. Similar structures were disclosed in the fructose series, where the bridges were between carbon atoms 2 and 6 in the normal forms and between 2 and 5 in those of the "γ" type. Goodyear and Haworth[23] suggested that the six atom ring type (1,5 bridge for glucose or 2,6 bridge for fructose) be given the class name *pyranose* and that the name *furanose* be used for the five atom ring forms. In order that such ring structures might be visualized readily from configurational formulas, Drew and Haworth[24] introduced general formulas that are shown here specifically in the D-glucopyranose series as Ia, Ib, IIa and IIb for the anomeric methyl D-glucopyranosides. Formula Ia is best understood if one arranges actual tetrahedra as represented in VII (or the equivalent XV) and forms the oxygen bridge connecting carbon atoms 1 and 5 of XV; it will be seen that tetrahedron 5 is best represented as turned so that the line showing the C_1—O—C_5 bridge (as in XXVI) lies below the plane of the paper and the dotting of this line indicates its low position. This turning of tetrahedron 5 is not a violation of the essence of Fischer's first convention though it may seem to be such by superficial consideration. This tetrahedron is one end of a series of several asymmetric carbon atoms and that apex of it which connects with asymmetric carbon atom 4, and an edge from this apex, remain in a straight line in the plane of the paper. This retention of the essence of Fischer's first convention is illustrated in XXVI, which is a more detailed representation of Ia. The essence of Fischer's first convention is that *all the apices which connect asymmetric carbon atoms shall lie in a straight line in the plane of the paper and this line shall contain lower edges of all these tetrahedra, their other apices being above the plane and on opposite sides of this line.* The formulas Ib and IIb (page 2) were introduced by Drew and Haworth[24] in order to give a clearer mode of expression of Ia and IIa by placing the six atom ring on its side and viewing it in perspective. The shading of the forward edges of the hexagon aids the visualization of the model and is especially helpful in introductory instruction.

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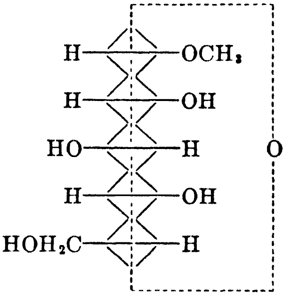
[21] W. J. Hickinbottom, *J. Chem. Soc.*, 3140 (1928); see also C. N. Riiber and N. A. Sørensen, *Kgl. Norske Videnskab. Selskabs, Skrifter*, No. 1 (1938).

[22] Consult W. N. Haworth's "The Constitution of Sugars," Edwin Arnold and Co., London (1929).

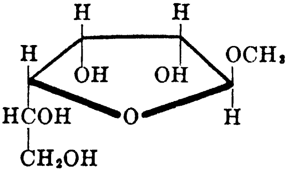
[23] E. H. Goodyear and W. N. Haworth, *J. Chem. Soc.*, 3136 (1927).

[24] H. D. K. Drew and W. N. Haworth, *J. Chem. Soc.*, 2305 (1926). Formulas embodying the general idea of this plan, but erroneously constructed, occur in an article by J. Böeseken, *Ber.*, **46**, 2622 (1913).

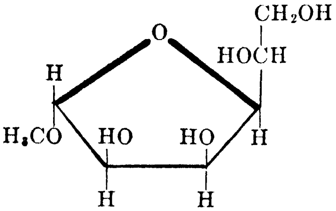


XXVI

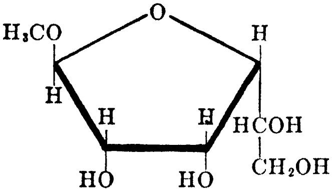
It is to be noticed that formulas I, Ia, II and IIa can be rotated in the plane of the paper without change in their significance but that this statement does not apply to such perspective formulas as Ib and IIb. If the perspective formula for methyl α-D-mannofuranoside (XXVII) is rotated 180° in the plane of the paper it becomes XXVIII, which can be regarded as a perspective formula of methyl β-D-gulofuranoside. To place the formula XXVII correctly in the desired position one rotates the actual *model* in the plane of the paper and draws the new perspective formula XXIX of the *model* in this new position. A simple rule to follow here is to observe that, with a perspective or three planar formula, rotation in the plane of the paper involves a rotation of three planes simultaneously. There is the plane of the ring itself, the plane below it in which are the OH and H addenda, and also the plane above the ring with similar addenda. To rotate such a perspective formula through 180° one must retain these three planes in their same relative positions. The addenda in the lowest plane will remain below the plane of the ring even when this is rotated, and similarly the addenda in the uppermost of the three planes will remain in their own plane. This is seen by referring to formulas XXVII and XXIX which are identical. The formula XXVIII is a false representation of XXVII because one has been confusing a plane projection formula with a three planar one.



XXVII



XXVIII

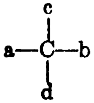


XXIX

The arrangements for the asymmetric carbon atom 5, which is outside the ring, are written in the customary form of Fischer's first convention.

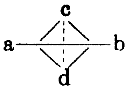
VII. Formulas Containing Only One Asymmetric Carbon Atom

It may seem that the formula for a substance containing only one asymmetric carbon atom should be less subject to misinterpretation than formulas involving many such atoms. However, such is not the case and the reason for this apparent anomaly is that the essence of Fischer's first convention, as expressed on p. 19, is not applicable unless there are two or more asymmetric carbon atoms in the structure. Consider the generalized formula XXX; is it to be interpreted as representing the model

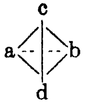


$$\begin{array}{c} c \\ | \\ a-C-b \\ | \\ d \end{array}$$

XXX

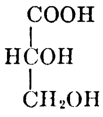


XXXI



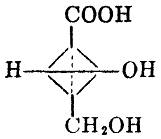
XXXII

XXXI or the model XXXII, which are enantiomorphs? Misinterpretation can be avoided in such cases if one uses the appropriate XXXI or XXXII, remembering that the dotted line represents the unseen edge of an opaque tetrahedron. The actual tetrahedron can be placed on the paper in six different positions corresponding to its six edges but the resulting projections represent the same enantiomorph without ambiguity if one uses a dotted line for the unseen edge. The formula XXXIII for D-glyceric acid is conventionally understood as referring to the model



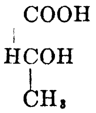
$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

XXXIII



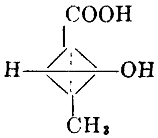
XXXIV

that is shown explicitly in XXXIV; the reason lies in the fact that the formula XVI for D-glyceraldehyde is well understood. The formula

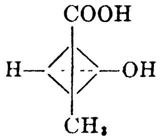


$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_3} \end{array}$$

XXXV



XXXVI

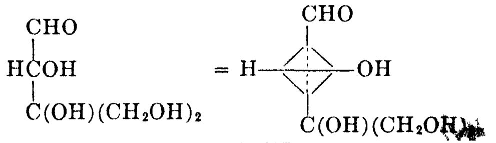


XXXVII

XXXV for the related levorotatory D-lactic acid is often misunderstood as implying XXXVII rather than the actual XXXVI.

VIII. Examples of Unusual Formulas from the Apiose Series

It has been shown by Schmidt[25] that the configurational aldehyde formula of apiose, the branched chain pentose which occurs in parsley, is XXXVIII.



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{C(OH)(CH_2OH)_2} \end{array}$$

XXXVIII

aldehydo-D-Apiose

By present conventions of nomenclature the mechanical model of apiose may be designated 3-bis(C-hydroxymethyl)-D-glyceraldehyde, and it is to be noted that only one asymmetric carbon atom is present in XXXVIII. The structure is not capable of assuming a pyranose ring but it can conceivably pass to tautomeric forms having the furanose ring and containing three asymmetric carbon atoms. There are four such configurations and they constitute the α and β anomers, respectively, of two different sugars, one of which is a 3-C-hydroxymethyl-D-erythrose and the other a 3-C-hydroxymethyl-L-threose. It is to be noticed in

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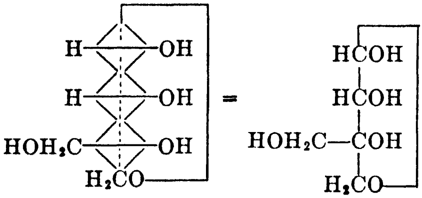
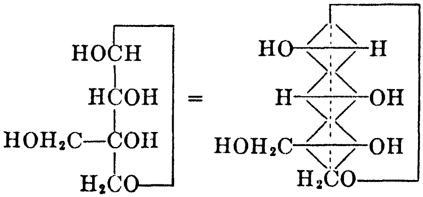
(25) O. T. Schmidt, *Ann.*, **483**, 115 (1930).

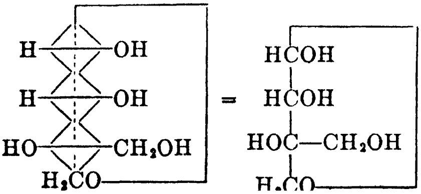
naming the anomers that α-D and β-L represent the same configuration of the anomeric carbon atom 1.[26] Neither apiose nor any one of its simple glycosides has ever been crystallized and therefore the formulas XXXIX to XLII are only illustrative of theory. Attention is called to them because they illustrate that the present conventional representation of mechanical models of carbohydrates, and the naming of such models, are free of ambiguity provided that one understands: (1) the essence of Fischer's first convention; (2) the Fischer-Rosanoff system of D and L nomenclature of carbohydrates; and (3) the writer's system of naming α and β anomers in the D and L carbohydrate series.



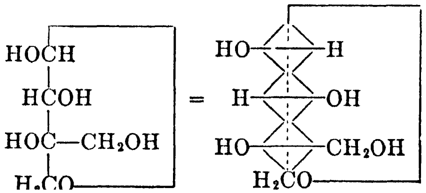
XXXIX
3-C-Hydroxymethyl-
α-D-erythrose



XL
3-C-Hydroxymethyl-
β-D-erythrose



XLI
3-C-Hydroxymethyl-
β-L-threose



XLII
3-C-Hydroxymethyl-
α-L-threose

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[26] The reader is invited to construct the models, formulas and names for two γ-lactones of apionic acid and for the four methyl apiohexofuranosides and eight methyl apiohexopyranosides that are indicated by theory as derivable from the hexoses that should result from the application of the cyanohydrin synthesis to apiose.

THE STRUCTURE AND REACTIVITY OF THE HYDRAZONE AND OSAZONE DERIVATIVES OF THE SUGARS

By E. G. V. Percival

The University of Edinburgh, Scotland

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I. Introduction

All carbohydrate chemists will agree that the first notable advance in the subject dates from the publication of the classical papers of Emil Fischer[1] on phenylosazone formation. Since that time the derivatives obtained by condensing sugars with phenylhydrazine and other substituted hydrazines have been used widely for purposes of identification and, in some cases, for the estimation and separation of sugars. With a few exceptions, however, it is only comparatively recently that attempts have been made to decide the precise structures of sugar hydrazones and osazones or that much attention has been given to the behavior of such compounds as chemical individuals. For that reason finality has only been reached on a few points and this review must therefore be considered as a progress report intended to show the general trend of work in this field.

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(1) E. Fischer, *Ber.*, **17**, 579 (1884); *ibid.*, **20**, 821 (1887)

Fischer represented the hydrazones and osazones as open chain structures; one sees at once, however, other structural possibilities since mutarotation occurs in solution in many cases. Unless this is due to decomposition, some form of tautomeric equilibrium must be postulated, and Haworth[2] pointed out that cyclic structures must be considered; Behrend and Lohr[3] recognized the possibility of the existence of *syn* and *anti* forms, and it has also been suggested[4] that there may be an equilibrium between hydrazone and azo structures in the osazones.

Information on these points has been sought by a study of rotation changes in solution, by the examination of derivatives and transformation products of the sugar hydrazones and osazones, and by synthetic experiments. While the conclusions drawn are not always in harmony with one another and the assignment of a particular structure to a derivative does not necessarily decide the constitution of the parent substance, many interesting observations have been made.

II. SUGAR HYDRAZONES

Except in a few cases such as D-mannose phenylhydrazone and D-galactose methylphenylhydrazone, the hydrazones are more difficult to isolate and are more unstable than the corresponding osazones. For these reasons, apart from the search for substituted hydrazones for identification purposes, the volume of published work on this subject is not great.

1. *The* D-*Glucose Phenylhydrazones*

In 1908, Behrend and Lohr[3] described the isolation of a "dextrose-β-phenylhydrazone," m. p. 141°, $[\alpha]_D$ $-5° \rightarrow -54°$ (water), which was different from the "dextrose-α-phenylhydrazone," m. p. 160°, $[\alpha]^{18}_D$ $-87° \rightarrow -54°$ (water). The "α" form was the original dextrose phenylhydrazone of Fischer, which had been isolated by Behrend[5] in a higher state of purity.[5a] From the rotational evidence it might have been concluded that these isomers represented true α and β forms, but Behrend and Lohr took the view that these "α" and "β" forms were either *syn* and *anti* forms of the true hydrazone, or that one of them was a true hydrazone (I) and the other a "hydrazide" (II).

Two years later Behrend and Reinsberg,[6] following the acetylation

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(2) W. N. Haworth, "The Constitution of Sugars," Edward Arnold and Co., London, p. 7 (1929).

(3) R. Behrend and F. Lohr, *Ann.*, **362**, 78 (1908).

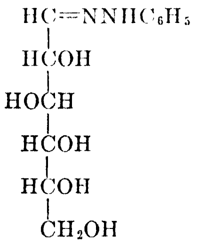
(4) E. Zerner and R. Waltuch, *Monatsh.*, **35**, 1025 (1914).

(5) R. Behrend, *Ann.*, **353**, 106 (1907).

(5*a*) The letters α and β have no structural significance.

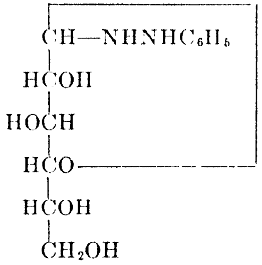
(6) R. Behrend and W. Reinsberg, *Ann.*, **377**, 189 (1910).

experiments of Hofmann,[7] showed that mild acetylation of the D-glucose "β"-phenylhydrazone gave an amorphous pentaacetate whereas D-glucose "α"-phenylhydrazone gave the crystalline pentaacetate, m. p. 152–3°, described by Hofmann, together with an amorphous pentaacetate which proved to be a mixture of the two forms. The crystalline "α"-pentaacetate was formulated as the cyclic compound III, although it should be noted that the assignment of a furanose ring is without experimental justification, and that the stereochemical arrangement of the hydrazide group is left undecided. Treatment with benzaldehyde and potassium hydroxide gave benzaldehyde acetylphenylhydrazone and hydrolysis with hydrochloric acid gave α-acetylphenylhydrazine. Furthermore when D-glucose was condensed with α-acetylphenylhydrazine and the product acetylated, the crystalline D-glucose "α"-phenylhydrazone pentaacetate, m. p. 152–3°, was obtained. Since no such evidence for the existence of an *N*-acetyl group could be found in the case of the amorphous "β" pentaacetate, the acyclic structure was proposed for this product and for the corresponding "β"-phenylhydrazone (I). These experiments on the D-glucose "α"-phenylhydrazone pentaacetate carried out more than thirty-five years ago constitute the only sound evidence of ring structure in any sugar hydrazone derivative.

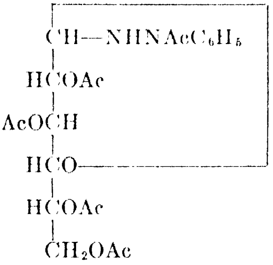


$$\begin{array}{c} HC{=}NNHC_6H_5 \\ | \\ HCOH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

I



II



III

Attempts have been made to solve the problem by examining the rate of hydrolysis of the two D-glucose phenylhydrazones, and Frèrejacque;[8] who used oxalic acid and picric acid to remove the phenylhydrazine from the sphere of the reaction as an insoluble salt, claimed to show that both of the D-glucose "α"- and "β"-phenylhydrazones are derived from α-D-glucose on account of the downward trend of the mutarotation observed on making the solutions alkaline. This result is therefore not in conflict with the conclusions of Behrend. From similar studies Stempel[9] concluded that "no information of value concerning

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(7) A. Hofmann, *Ann.*, **366,** 305 (1909).

(8) M. Frèrejacque, *Compt. rend.* **180,** 1210 (1925)

(9) G. H. Stempel, Jr., *J. Am. Chem. Soc.*, **56,** 1351 (1934)

either the structures of the D-glucose phenylhydrazones or their relationships to the isomers of D-glucose can be obtained from hydrolysis studies of the hydrazones." In a later contribution[10] the view is expressed that the formation of D-glucose phenylhydrazone is due to a reaction between $C_6H_5NH{\cdot}NH_3^+$ and an intermediate substance which is symmetrically related to the isomeric forms of D-glucose. It is clear that there is no evidence for presuming the D-glucose "α"- and "β"-phenylhydrazones to be derived from α- and β-D-glucose.

2. *The* D-*Fructose Methylphenylhydrazones*

A further example of isomerism in the hydrazone series may be mentioned. Two D-fructose methylphenylhydrazones are known; one,[11] m. p. 118–119°, $[\alpha]^{22}_{D} \pm 0°$; and another,[12] m. p. 170°, $[\alpha]^{18}_{D} - 253°$ (pyridine-alcohol). This latter substance gave a crystalline pentaacetate, m. p. 121°, $[\alpha]^{17}_{D} + 86.5°$ (chloroform) whereas Ofner's methylphenylhydrazone gave a sirupy pentaacetate[13] $[\alpha]^{22}_{D} - 75°$ (chloroform). On the basis of the large negative rotation of the D-fructose methylphenylhydrazone, m. p. 170°, and the negligible value of Ofner's product, the suggestion has been made that the former may be cyclic and the latter acyclic. This point has not, so far, been clarified although it has been demonstrated[14] that treatment with phenylhydrazine yields two isomeric D-glucose phenyl-methylphenylosazones. This point will be referred to later (Part III).

3. *The Acyclic Hydrazones of* D-*Galactose*

Much more clear-cut evidence is available from the work of Wolfrom and coworkers, as to the structure of the phenylhydrazone and substituted phenylhydrazones of D-galactose. Wolfrom and Christman[15] showed that the ethyl hemiacetal of *aldehydo*-D-galactose pentaacetate (IV) on treatment with phenylhydrazine, gave the same crystalline product (V) as the pentaacetate obtained by the mild acetylation of D-galactose phenylhydrazone (VI). D-Galactose phenylhydrazone must therefore be regarded as acyclic, if we set aside the possibility of the migration of acetyl groups. Similar results were obtained for the benzylphenyl-, methylphenyl-, and *p*-nitrophenylhydrazones of D-galactose.

Full support for this view was afforded by the experiments of Compton

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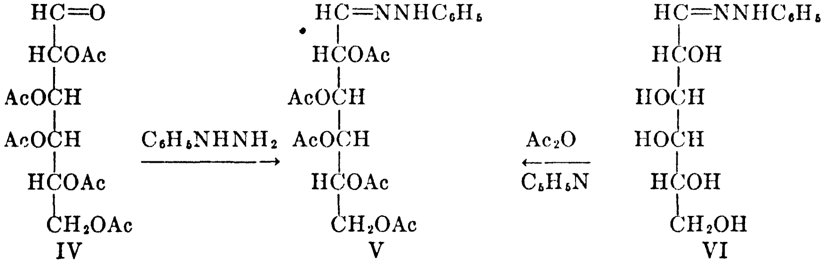
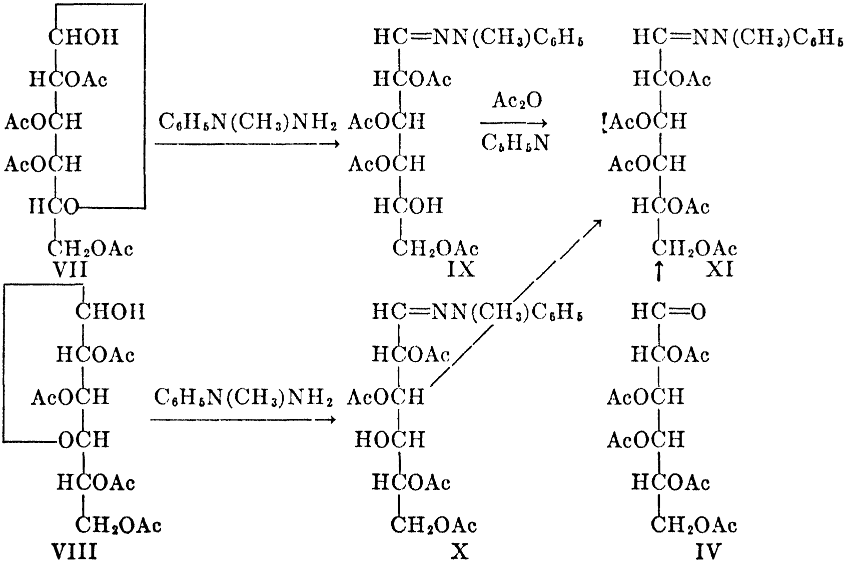
(10) A. Orning and G. H. Stempel, Jr., *J. Org. Chem.*, **4**, 410 (1939).
(11) R. Ofner, *Monatsh.*, **26**, 1165 (1905).
(12) Elizabeth E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 1320 (1937).
(13) W. J. Heddle and E. G. V. Percival, *J. Chem. Soc.*, 1511 (1940).
(14) Elizabeth E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 750 (1941).
(15) M. L. Wolfrom and C. C. Christman, *J. Am. Chem. Soc.*, **53**, 3413 (1931).

and Wolfrom[16] who showed that D-galactose 2,3,4,6-tetraacetate (VII) and D-galactose 2,3,5,6-tetraacetate (VIII) could be converted into the corresponding phenylhydrazones and also into the methylphenylhydrazones IX and X, respectively.



Both tetraacetyl-D-galactose phenylhydrazones on mild acetylation gave the pentaacetyl-D-galactose phenylhydrazone (V) previously obtained by Wolfrom and Christman, and the two corresponding tetraacetyl-D-galactose methylphenylhydrazones IX and X gave the acyclic pentaacetyl-D-galactose methylphenylhydrazone (XI) of those authors.



That migration of acetyl groups had not taken place during the formation of the D-galactose methylphenylhydrazone tetraacetates was shown by the isolation of two different *p*-toluene sulfonates from IX and X. This evidence shows therefore that even when oxide rings are present in the original compounds, as soon as the hydrazones are formed, the open-chain structure is produced. Since no isomeric forms are known in the D-galactose hydrazone series the present view is therefore that these substances are open-chain forms.

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[16] J. Compton and M. L. Wolfrom, *J. Am. Chem. Soc.*, **56**, 1157 (1934).

4. *Optical Properties of Sugar Hydrazones*

In 1917, Hudson[17] pointed out an interesting relationship between the sign of the optical rotations of eleven sugar benzylphenylhydrazones; namely that when the hydroxyl group on carbon atom 2 was to the right in the projection formula, as in D-glucose, the specific rotations were negative, whereas the values were positive when the corresponding hydroxyl group was on the left, as in D-mannose. The optical rotations of the benzylphenylhydrazones are thus dominated by the configuration of carbon atom 2 in the same way as for the phenylhydrazides of the sugar acids, although in the opposite sense.

Votoček and coworkers[18] confirmed this result and extended it to the *p*-chlorobenzylphenyl-, benzyl-*p*-tolyl- and dibenzyl-hydrazones which were found to behave in the same way as the benzylphenylhydrazones. On the other hand the ethylphenyl-, butylphenyl- and diphenyl-hydrazones as well as the phenyl-β-phenylethylhydrazones do not follow the rule; the presence of a *benzyl* group or substituted benzyl group seems to be the deciding factor.

III. STRUCTURAL STUDIES ON SUGAR OSAZONES

Although, as mentioned previously, other possibilities of isomerism exist, most attention has been devoted to attempts to decide whether sugar osazones are cyclic or acyclic. The results of methylation experiments indicate that under these conditions the osazones react as cyclic structures, but there is less certainty about the structure of acetylated osazones.

During the initial stages of the methylation of D-glucose phenylosazone[19] a crystalline monomethyl-D-glucose phenylosazone, recognized as 5-methyl-D-glucose phenylosazone was isolated, indicating that the hydroxyl group on C_5 was not involved in an oxide ring. By the complete methylation of D-glucose phenylosazone a substance originally described as a trimethyl-D-glucose phenylosazone (later corrected to a trimethyl-D-glucose methylphenylphenylosazone[20]) was obtained. This substance

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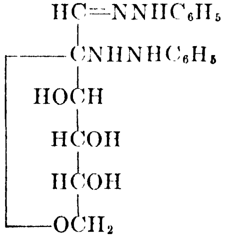
(17) C. S. Hudson, *J. Am. Chem. Soc.*, **39**, 462 (1917).

(18) E. Votoček, F. Valentin and O. Leminger, *Collection Czechoslov. Chem. Commun.*, **3**, 250 (1931); E. Votoček and Z. Allan, *ibid.*, **8**, 313 (1936); E. Votoček and O. Wichterle, *ibid.*, **8**, 322 (1936).

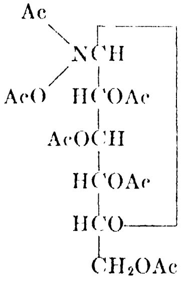
(19) Elizabeth E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 1398 (1935).

(20) Elizabeth E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 1320 (1937).

gave rise to a trimethyl sugar recognized as 3,4,5-trimethyl-D-fructose since tetramethyl-D-fructopyranose was produced on further methylation. These facts led to the suggestion that D-glucose phenylosazone was in fact D-fructopyranose phenylosazone (XII). Support for this view was afforded by the observation[21] that D-glucose phenylosazone failed to react with triphenylmethyl chloride, a reagent which normally reacts readily with primary alcohol groups. A similar result was obtained with D-galactose phenylosazone[22] from which on complete methylation a crystalline trimethyl-D-galactose methylphenylphenylosazone was obtained, no more methoxy groups entering the molecule. Accordingly D-galactose phenylosazone has been designated as D-tagatopyranose phenylosazone. Contrary views have been expressed by Engel[23] who also carried out methylation experiments with D-glucose phenylosazone. It is clear from his paper, however, that the highest methoxyl content which he was able to reach (20.7%) closely approached that for a trimethyl-D-glucose methylphenylphenylosazone (22.5%), whereas for a tetramethyl-D-glucose phenylosazone a value of 29.0% should have been obtained. This result therefore is not in conflict with the cyclic structure. The main evidence brought forward by Engel in support of an open chain formula for the sugar osazones is the similarity in the absorption spectra of a series of sugar osazones and osazone derivatives including D-glyceraldehyde phenylosazone, which would not be expected to be cyclic. This author points out, however, that the absorption spectra of the sugar osazones differ markedly from those of the dihydrazones of glyoxal and methylglyoxal and attributes this difference to the effect of the oxygen atom on C_3 in the former series. This result is open to other interpretations, however. In addition, Engel seeks to show that the apparent mutarotation of many sugar osazones in solution is due to hydrolysis.



XII



XIII

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(21) O. Diels, E. Cluss, H. J. Stephan and R. König, *Ber.*, **71**, 1189 (1938).

(22) J. R. Muir and E. G. V. Percival, *J. Chem. Soc.*, 1479 (1940).

(23) L. L. Engel, *J. Am. Chem. Soc.*, **57**, 2419 (1935).

He shows that D-fructose methylphenylosazone in contact with phenylhydrazine at room temperature yields D-fructose methylphenylphenylosazone and with *p*-nitrophenylhydrazine, D-fructose methylphenyl-*p*-nitrophenylosazone. It is possible to interpret this result in another way, namely that since the competing base removes one methylphenylhydrazine residue but not both, one is less firmly bound than the other, and might be admitted as evidence that the two methylphenylhydrazine residues are bound by different types of linkage to the sugar, as in fact the cyclic formulation demands.

The tetraacetates of D-glucose phenylosazone and D-galactose phenylosazone have been examined[24] using a method of deacetylation which had been used with success to determine acetoxy groups in alkali labile sugar acetates.[25] Applying this method to the cyclic "β"-D-glucose oxime hexaacetate (XIII) it was possible to show that five O-acetyl groups and one N-acetyl group were present, the latter not suffering hydrolysis under the experimental conditions.

The four acetyl groups of the hexose phenylosazone tetraacetates when examined in this way all appeared to be joined through oxygen, and Wolfrom and coworkers concluded, therefore, that these osazone acetates are open-chain compounds.

Some doubts have been expressed[20] as to whether such a conclusion is justified, on the ground that, if the osazone acetates are cyclic, the fourth acetyl group would be present in an acetylated hydrazide and could not be compared with the acetyl group in such a compound as acetanilide or methylacetanilide. In α,β-diacetylphenylhydrazine, for example, an amount of acetyl corresponding to about one acetyl group is removed under conditions which would be expected to saponify only O-acetyl groups.

To sum up, while the balance of the evidence indicates that the osazone acetates examined are probably acyclic, the possibility that cyclic acetates exist cannot at present be discarded.

The oxidation of D-glucose phenylosazone by periodic acid has been shown to yield 1-phenyl-4-phenylhydrazono-5-pyrazolone by the intermediate formation of the 1,2-bis(phenylhydrazone) of mesoxaldehyde.[25a] The reaction proceeds normally as if the osazone were acyclic; it must be presumed, therefore, that there is an equilibrium between the cyclic (XII) and acyclic forms in the aqueous-ethanolic solutions employed,

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(24) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *J. Am. Chem. Soc.*, **58,** 490 (1936).

(25) A. Kunz and C. S. Hudson, *J. Am. Chem. Soc.* **48,** 1982 (1926).

(25*a*) P. Karrer and K. Pfaehler, *Helv. Chim. Acta*, **17,** 766 (1934). E. Chargaff and B. Magasanik, *J. Am. Chem. Soc.*, **69,** 1459 (1947).

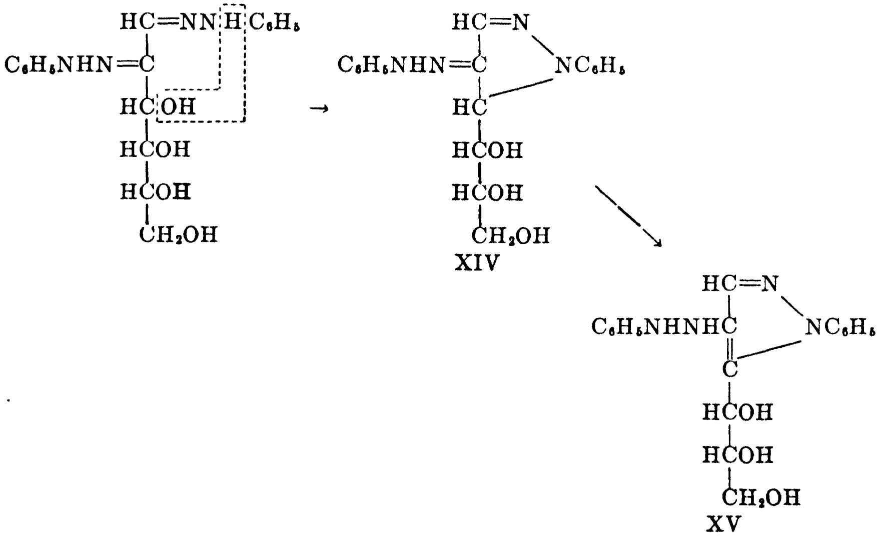
such as exists, for example, in solutions of D-glucopyranose, which is also completely disrupted by periodic acid. The stability of the pyranose ring in XII must be compared, therefore, with the pyranose ring in a reducing sugar rather than in a glycoside, which would explain the apparently conflicting results discussed above.

IV. Anhydrides of Osazones and Hydrazones

1. *Anhydrides Prepared Directly from the Corresponding Osazones*

Although Fischer[1] described a lactose phenylosazone anhydride in 1887, little attention was paid to substances of this type until 1935 when Diels and Meyer[26] reported the isolation of monoanhydrides of D-glucose phenylosazone, D-galactose phenylosazone, D-xylose phenylosazone, L-arabinose phenylosazone, lactose phenylosazone, and cellobiose phenylosazone, as well as a dianhydride of maltose phenylosazone, by boiling the corresponding osazones in alcoholic solution with a little sulfuric acid. Because of the apparent identity of the D-glucose phenylosazone anhydride with the 3,6-anhydro-D-glucose phenylosazone of Fischer and Zach,[27] Diels and Meyer formulated these compounds as 3,6-anhydrides.

This formulation was abandoned in a later paper[28] in favor of a pyrazole structure (XV) produced by way of a 4-pyrazolone derivative (XIV), as follows. The authors interpret the production of the same


XIV

XV

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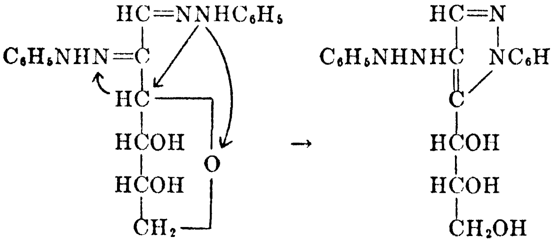
(26) O. Diels and R. Meyer, *Ann.*, **519**, 157 (1935).

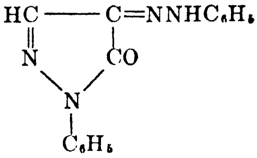
(27) E Fischer and K. Zach, *Ber.*, **45**, 456 (1912); H. Ohle, L. v. Vargha and H. Erlbach, *ibid.*, **61**, 1212 (1928).

(28) O. Diels, R. Meyer and O. Onnen, *Ann.*, **525**, 94 (1936).

D-glucose phenylosazone anhydride from 3,6-anhydro-D-glucose phenylosazone (XVI) in the following manner. In support of this view it is



XVI → XV



XVII

pointed out that anhydro-osazones cannot be caused to form osones and that anhydro-osazones are not produced when alkylphenylosazones are used. The authors also cite the production of 5-keto-4-phenylhydrazono-1-phenyl-4,5-dihydropyrazole (XVII), by boiling both the osazones and the monoanhydrides with hydroxylamine hydrochloride in methanol, as evidence that anhydride formation involves interaction between groups attached to C_1 and C_3, although the mechanism of the reaction is not understood. It has now been shown[29] that XV does not represent the true structure of the monoanhydro-osazones of Diels and coworkers.

In 1937 it was demonstrated[20] that the monoanhydro derivatives of D-glucose phenylosazone and D-galactose phenylosazone prepared by Diels formed diacetates and not triacetates, a fact which points to the presence of a second ring system. That the hydroxyl group on C_6 is involved is now shown by the fact that the D-glucose phenylosazone anhydride failed to react with triphenylmethyl chloride nor did its *p*-toluenesulfonate react with sodium iodide in acetone. Failure to condense the product with acetone eliminates either the structure proposed by Diels (XV) or a structure where free hydroxyl groups are present on C_5 and C_6 or C_4 and C_5. For these reasons structure XVIII is proposed,[29] the production from the cyclic D-fructopyranose phenylosazone having involved the loss of water from the hydrazide residue on C_2 and the hydroxyl group on C_6.

In addition, it has been shown that Diels' D-glucose phenylosazone anhydride is not identical with 3,6-anhydro-D-glucose phenylosazone for although the osazones are very similar, the diacetates have widely different properties.

Again no free primary alcohol residue could be detected in the phenylosazone prepared directly from 3,6-anhydro-D-glucose, no reaction taking place with triphenylmethyl chloride or even with *p*-toluenesulfonyl

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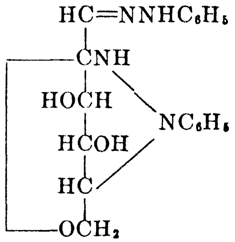
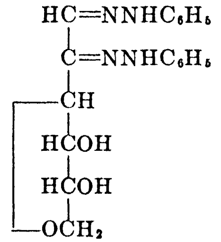
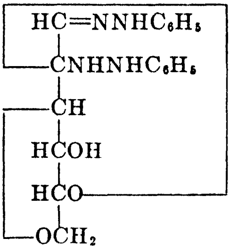
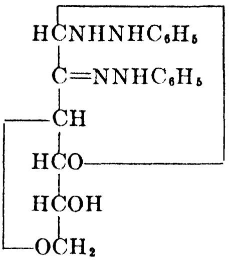
(29) E. G. V. Percival, *J. Chem. Soc.*, 783 (1945).

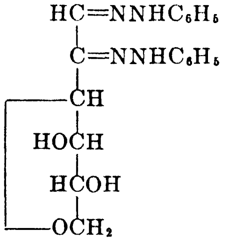
chloride. Although there would seem to be no reason why the 3,6-anhydro ring should not be retained in 3,6-anhydro-D-glucose phenylosazone, structure XIX is inadmissible since condensation with acetone could not be effected. Formulas XX and XXI are therefore suggested for this substance although a decision between them has not been made.

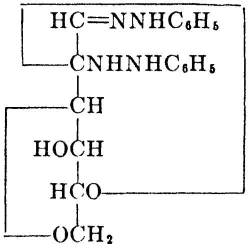

XVIII


XIX


XX


XXI

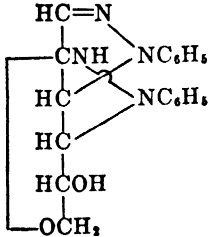

XXII


XXIII

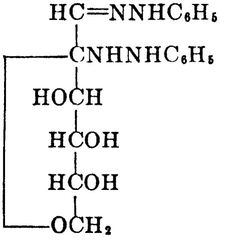
On the other hand, the monoanhydride prepared from D-galactose phenylosazone by Diels is certainly identical with the osazone prepared from 3,6-anhydro-D-galactose. In this case too, formula XV proposed by Diels cannot be sustained since the compound yields a diacetate, fails to react with triphenylmethyl chloride (or *p*-toluenesulfonyl chloride) or to condense with acetone. When the diacetate is deacetylated, an isomeric D-galactose phenylosazone anhydride, m. p. 226°, is obtained, also with no free primary alcoholic group. It is considered likely that the original 3,6-anhydro ring is retained in both these products and formulas XXII and XXIII would fit the facts. The analog of XXI is not possible in the galactose series because of the stereochemical arrangement of the groups on C_4. The possibility that *syn* and *anti* isomerism is concerned cannot be excluded, however.

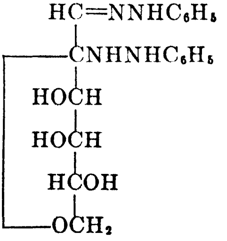
2. *Anhydrides Prepared by the Deacetylation of Acetates*

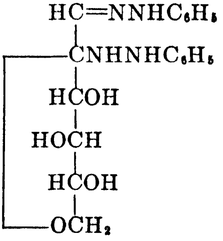
The discovery was made in 1936 that by deacetylating D-glucose phenylosazone and D-galactose phenylosazone tetraacetates at room temperature[30] both compounds gave the same product, a hexose phenylosazone dianhydride, which yielded a monoacetate and a monomethyl ether. This substance was stable enough to be recrystallized from concentrated hydrochloric acid. It was formulated as XXIV. Later,[31] the absence of a primary alcohol residue on C_6 was confirmed by the failure of the *p*-toluenesulfonate to react with sodium iodide, and by the isolation of the same hexose phenylosazone dianhydride from D-gulose phenylosazone tetraacetate and of its enantiomorph from L-sorbose phenylosazone tetraacetate.


XXIV

If one considers the formulation of D-glucose phenylosazone (XXV), D-galactosephenylosazone (XXVI) and D-gulose phenylosazone (XXVII) as ketopyranose phenylosazones, it is clear that the only way in which the same dianhydride can be produced from all three is by reactions involving the hydroxyl groups on C_3 and C_4 since the configuration of C_5 is the only common factor. It would appear that Walden inversion can take place on C_3 or C_4 or on both in order to arrive at the most stable configuration of the three rings, but the stereochemical configuration of the hexose phenylosazone dianhydride has not yet been decided.


XXV


XXVI


XXVII

Osazone anhydrides have also been prepared by the deacetylation of the tetraacetates of D-galactose methylphenyl-phenylosazone and from

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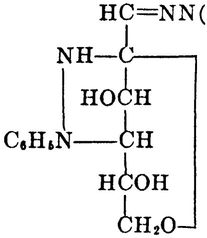
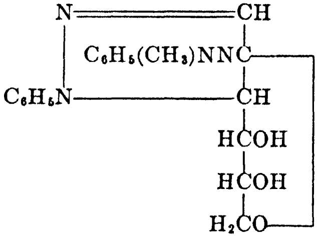
(30) E. G. V. Percival, *J. Chem. Soc.*, 1770 (1936).
(31) E. G. V. Percival, *J. Chem. Soc.*, 1384 (1938).

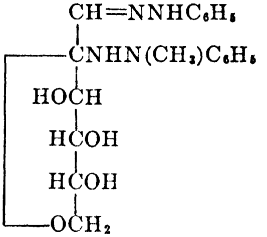
the two isomeric D-glucose methylphenyl-phenylosazones, the structures proposed being XXVIII and XXIX.[14]



XXVIII



XXIX



XXX

Since the methylphenylhydrazone residue cannot participate in anhydride formation, the products are monoanhydrides. Nevertheless, they contain only two free hydroxyl groups since they yield diacetates; therefore, oxide rings must be present in these derivatives, and since it was proved in both cases that no primary alcohol group was present, the ketopyranose ring is postulated. The allocation of the point of attachment of the anhydro ring to C_4 instead of C_5 in the D-galactose derivative (XXVIII) depends on the fact that it would not condense with acetone and the structure of the corresponding D-glucose derivative (XXIX) was established by the isolation of a crystalline monoacetone compound.

With regard to the isomeric D-glucose methylphenyl-phenylosazones from which XXIX was prepared, it may be recalled that Votoček and Vondráček[32] treated D-glucose phenylhydrazone with methylphenylhydrazine and obtained two osazones: A, m. p. 192°; and B, m. p. 205°. B was also obtained from D-glucose methylphenylhydrazone and phenylhydrazine. A was described as D-glucose phenyl-methylphenyl-osazone and B as D-glucose methylphenyl-phenyl-osazone. These experimental facts were verified and the product A was found to have $[\alpha]^{17}\text{D} - 53° \rightarrow -6°$ (pyridine-alcohol) and B $[\alpha]^{17}\text{D} - 60° \rightarrow -15°$. A new interpretation has since been put on these observations[14] because it was found that the D-fructose methylphenylhydrazone of Ofner,[11] m. p. 118°, on treatment with phenylhydrazine gave B, whereas the D-fructose methyl-

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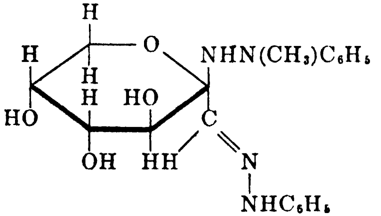
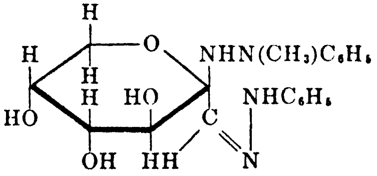
(32) E. Votoček and R. Vondráček, *Ber.*, **37**, 3848 (1904).

phenylhydrazone of m. p. 170° gave A. Since the formation of the osazones was about seven times as fast from the D-fructose methylphenylhydrazones as when the D-glucose derivatives were used, and since no mixtures were formed in the process, it was considered that both A and B carried the methylphenylhydrazine residue on C_2, that is, they were both D-glucose phenyl-methylphenylosazones, depicted as a D-fructopyranose derivative in XXX. A and B are therefore to be regarded as isomers, but the isomerism is unlikely to be due to a different stereochemical (α,β) arrangement since the rotations are so nearly identical. Furthermore the tetraacetates (amorphous) obtained on acetylation were indistinguishable from one another and on deacetylation yielded the same anhydride (XXIX), the structure of which has already been discussed. It is possible therefore that we have here an example of geometric isomerism involving *syn* and *anti* forms such as XXXI and XXXII.



XXXI



XXXII

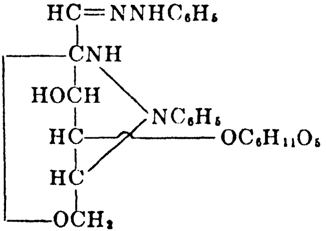
For structure XXXI, anhydride formation on C_3 is impossible even allowing for the possibility of Walden inversion; this can be seen readily by the use of models. In order to explain the production of the same anhydride XXIX from both, it is necessary to suppose that the *syn* form undergoes conversion to the *anti* form (XXXII) at some stage.

3. *Disaccharide Anhydro-osazones*

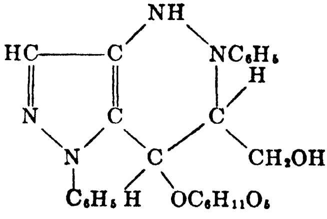
Cellobiose phenylosazone when treated by Diels' method yielded a monoanhydride in the form of a hydrate. The same product was also obtained from cellobiose phenylosazone heptaacetate by deacetylation.[19] The anhydride gave a pentaacetate proving that an oxide ring system was present, and structure XXXIII was proposed although the possibility of 1,3-anhydro- (1,5-oxide), 1,3-anhydro- (2,6-oxide) and 1,3-anhydro- (2,5-oxide) structures could not be rigidly excluded.

Lactose phenylosazone heptaacetate yielded lactose phenylosazone anhydride hydrate,[20] a compound previously isolated by Fischer[1] and by Diels and Meyer.[26] This compound also yields a pentaacetate; the same structural possibilities occur here as in the case of the cellobiose phenylosazone anhydride mentioned above.

Maltose phenylosazone heptaacetate, on the other hand, yielded two isomeric maltose phenylosazone anhydrides[20] on deacetylation, both of which gave rise to pentaacetates; like the other disaccharide anhydro-osazones, their precise structures are not known. It may be recalled that Diels and Meyer[26] isolated a dianhydride of maltose phenylosazone



XXXIII

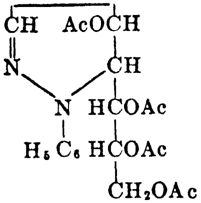


XXXIV

which they tentatively formulated as a compound containing a condensed pyrazolopyridazine nucleus XXXIV; this serves to emphasize the differences between anhydride formation by the two methods.

4. D-*Mannose Anhydrophenylhydrazone Tetraacetate*

Wolfrom and Blair[32a] have reported a crystalline product obtained by the acetylation of D-mannose phenylhydrazone with acetic anhydride and pyridine. Four easily saponifiable groups led to their allocation by these workers as O-acetyl.[24] While no definitive structure could be assigned on the evidence available, the analytical data inclined the authors to favor a pyrazoline structure (XXXIVa); other possibilities obviously exist.



XXXIVa

V. THREE IMPORTANT REACTIONS OF OSAZONES

1. *Conversion of Osazones into Osotriazoles*

An outstanding contribution to osazone chemistry was made by Hann and Hudson[33] who examined the effect of boiling osazones with

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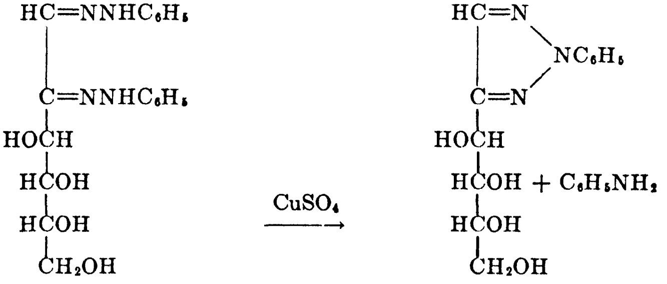
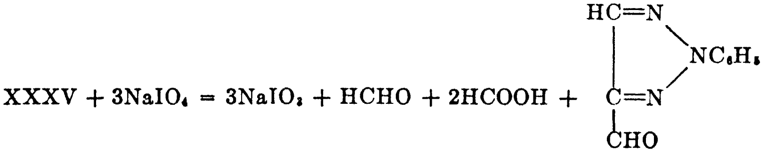
(32a) M. L. Wolfrom and Mary G. Blair, *J. Am. Chem. Soc.*, **68**, 2110 (1946).

(33) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 735 (1944).

copper sulfate solution. With D-glucose phenylosazone, the sparingly soluble D-glucose phenylosotriazole (XXXV) was isolated in a yield of nearly 70%, aniline being eliminated during the reaction. This product,


XXXV

m. p. 195–196°, $[\alpha]^{20}D - 81.6°$ (pyridine), affords a valuable test for the presence of small quantities of D-glucose, and was shown to be 2-phenyl-4-[D-*arabino*-tetrahydroxybutyl]-2,1,3-triazole by the following reactions. It formed a crystalline tetraacetate and tetrabenzoate. Oxidation with sodium periodate proceeded almost quantitatively according to the following scheme to give the previously known 2-phenyl-4-formyl-2,1,3-triazole (XXXVI) in 84% yield. Good yields of the corresponding osotriazoles were obtained[34] from the phenylosazones of L-sorbose, D-galactose, D-altrose, D-xylose, D-, L- and D,L-arabinose, L-rhamnose, L-fucose, D-atroheptose, D-mannoheptose, D-galaheptose, lactose, cellobiose, melibiose and turanose.[35] While the mechanism of this interesting reaction is not yet clear, it appears that a red osazone-copper ion complex is first formed and then decomposes to give aniline and the very stable osotriazole. That the reaction is a general one for the sugar osazones is emphasized in the later publications in which the constitution of various phenylosotriazoles is proved by periodate oxidation. It should be noted that the absence of a ring structure in the osotriazoles does not prove or disprove the absence of an oxide ring in the original osazones.


XXXVI

When a stream of air or oxygen is passed through an alkaline solution of a phenylosazone, Diels and coworkers[21] showed that oxidation takes place with the loss of two hydrogen atoms and the production of a dehydrophenylosazone. Like the parent hexose phenylosazones these compounds failed to react with triphenylmethyl chloride and yielded triacetates; in a similar manner the disaccharide dehydrophenylosazones yielded hexaacetates. The 2,6-oxide ring structure is therefore presumed to be present in these compounds and Diels formulates the dehydrophenylosazone of D-glucose as an osotriazole derivative.

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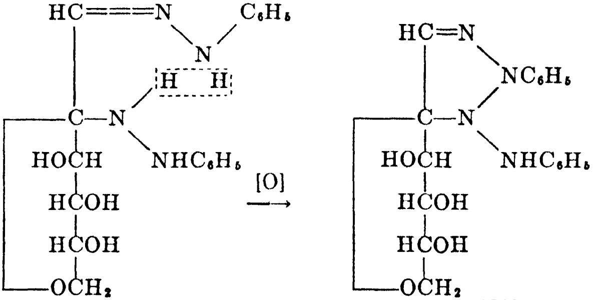
[34] W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **67**, 939 (1945); **68**, 1766 (1946); **69**, 1050, 1461 (1947).

[35] C. S. Hudson, *J. Org. Chem.*, **9**, 470 (1944).



XXXVII

It was found impossible to prepare dehydrophenylosazones from methylphenylosazones or from the monoanhydrophenylosazones prepared by the sulfuric acid method, in agreement with the view that the dehydrogenation was concerned with the substituents on C_1 and C_2. It has been shown[29] that deacetylation of 3,6-anhydro-D-glucose phenylosazone diacetate in air yields a dehydroanhydrophenylosazone, but that this reaction does not take place in an atmosphere of nitrogen. This result is in harmony with the two alternative structures XX and XXI proposed for 3,6-anhydro-D-glucose phenylosazone, and an examination of the formula proposed for the D-glucose phenylosazone anhydride prepared by Diels' method (XVIII) shows why this derivative cannot be dehydrogenated in this way. Similar considerations hold for the anhydro-D-galactose phenylosazone XXII but not for XXIII. This negative evidence would therefore appear to favor XXII for the D-galactose phenylosazone anhydride prepared both by Diels' process and directly from 3,6-anhydro-D-galactose.

2. *Hydrogenation of Osazones*

Fischer[36] reduced D-glucose phenylosazone to "iso-D-glucosamine" (XXXVIII) using zinc and acetic acid. More recently catalytic hydrogenation has been applied[37] with improved results, D-glucose phenylosa-

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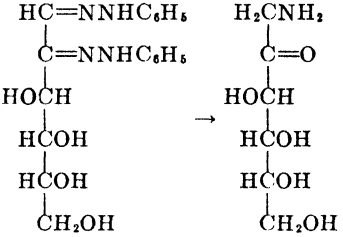
(36) E. Fischer, *Ber.*, **19**, 1920 (1886).

(37) K. Maurer and B. Schiedt, *Ber.*, **68**, 2187 (1935).

zone giving "iso-D-glucosamine" (as the acetate) in 60% yield using a palladium catalyst in acetic acid. It is pointed out that the catalytic hydrogenation of sugar osazones and phenylhydrazones proceeds differently from that of simple hydrazones, phenylhydrazones and phenylosazones, which always give hydrazo compounds without cleavage of the molecule. Thus acetophenone ketazine, $C_6H_5(CH_3)C{=}N{-}N{=}C(CH_3)C_6H_5$ gives



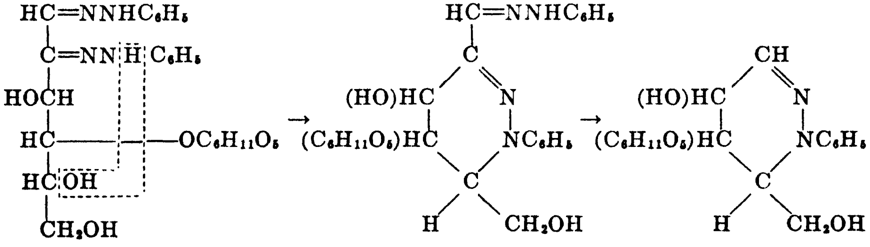
$$\begin{array}{c} HC{=}NNHC_6H_5 \\ | \\ C{=}NNHC_6H_5 \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array} \rightarrow \begin{array}{c} H_2CNH_2 \\ | \\ C{=}O \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

XXXVIII

1,2-bis(α-methylbenzyl) hydrazine ($C_6H_5(CH_3)CHNHNHCH(CH_3)C_6H_5$) instead of α-methylbenzylamine, $C_6H_5(CH_3)CH{\cdot}NH_2$, and benzil phenylosazone is unchanged under the conditions of hydrogenation described for D-glucose phenylosazone. The conclusion is drawn that the adjacent hydroxyl groups influence the behavior of the sugar osazones, but it is more probable that the oxide ring structure is concerned in some way.

3. *Action of Bases on Osazones*

Diels, Meyer and Onnen[28] studied the effect of boiling alcoholic solutions of osazones with potassium hydroxide (1%). D-Glucose phenylosazone gave glyoxal phenylosazone and 1,2-bis(phenylazo) ethylene, and in general, glyoxal phenylosazone was obtained in the other cases examined, except for cellobiose phenylosazone. Evidently fission takes place between C_2 and C_3 by the action of alkali. Cellobiose phenylosazone is abnormal and it is considered that a pyridazine derivative XXXIX is produced. Maltose phenylosazone, on the other hand, behaves normally to give glyoxal phenylosazone; it may be recalled that cellobiose



XXXIX

phenylosazone reacts normally with acids to give a monoanhydride whereas maltose phenylosazone gives a dianhydride under the same conditions.

VI. Theories of Osazone Formation

It is generally assumed that in the course of osazone formation the phenylhydrazine removes two hydrogen atoms from the carbon chain of the phenylhydrazone which is formed first, thus producing a keto group (from an aldose) or an aldehyde group (from a ketose). Since phenylhydrazine is normally a strong reducing agent this alleged behavior is very remarkable and it is not surprising that other explanations have been sought.

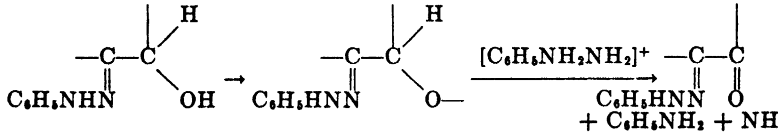
Kenner and Knight[38] point out that hydrazones are formed from free hydrazine bases, whereas hydrazine salts, such as phenylhydrazine acetate, yield osazones. It is emphasized that salt formation greatly favors such reactions as the production of amines from hydrazo compounds, a reaction which is represented as follows. The cation therefore

$$C_6H_5NHNHC_6H_5 \xrightarrow{H^+} [C_6H_5NH_2NHC_6H_5]^+ \xrightarrow{e\,+\,H} 2C_6H_5NH_2$$

acts as an oxidizing agent. Phenylhydrazine salts were tested as oxidizing agents using the free base as the reducing agent and it was shown that the free base required a temperature of 300° for decomposition whereas the following reaction took place readily at 165°. In harmony with this

$$[C_6H_5NH_2NH_2]^+ + C_6H_5NHNH_2 \rightarrow C_6H_5NH_2 + NH_3 + C_6H_6 + N_2 + H^+$$

view is the fact that nitro groups would be expected to increase the demands of the hydrazine salt cations for electrons and so increase the oxidizing power, whereas alkyl groups would weaken it. It is considered that this explains why nitrophenylosazones are produced with great ease at room temperature[39] and that methylphenylhydrazine under normal conditions reacts only with ketoses to give methylphenylosazones[40]; in other words, it oxidizes only primary alcohol residues. The course of osazone formation is depicted according to Kenner and Knight[38] as follows. There is some evidence[10] that the careful exclusion



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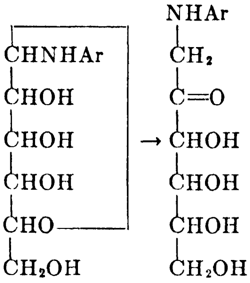
(38) J. Kenner and E. C. Knight, *Ber.*, **69**, 341 (1936).

(39) A. Reclaire, *Ber.*, **41**, 3665 (1908).

(40) C. Neuberg, *Ber.*, **37**, 4616 (1904).

of dissolved oxygen from the reaction mixture inhibits osazone formation at 30°, but this is rather far removed from the usual experimental conditions; too much weight should not be given to this observation in considering a reaction mechanism for osazone formation.

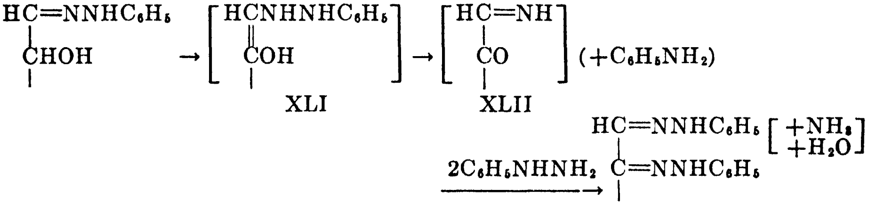
An ingenious theory has been advanced by Weygand[41] based on the observation that when aryl (*p*-tolyl-, *p*-phenetyl-) isoglycosamines (XL), produced from the corresponding nitrogen glycosides by the Amadori rearrangement, are heated with phenylhydrazine hydrochloride and



XL

sodium acetate, the yield of osazone is very much higher than when the corresponding sugar is used. Even if the arylisoglycosamine is not actually isolated, but *p*-toluidine or *p*-phenetidine is added to a solution of the sugar, phenylhydrazine hydrochloride, and sodium acetate, high yields of the phenylosazone result.

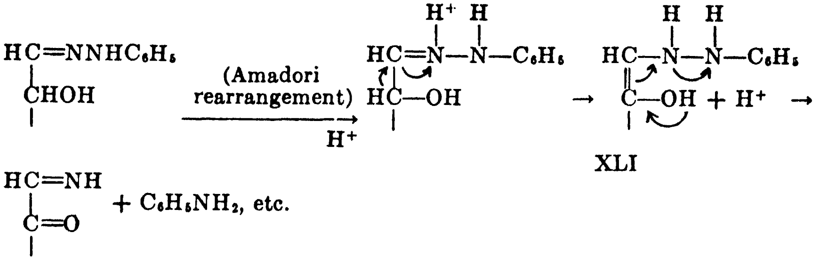
Two possible routes are suggested for osazone formation under the usual conditions. The first involves formation of the phenylhydrazone, isomerization to an enolic form XLI followed by the loss of aniline to form an imino ketone XLII (or an imino aldehyde from a ketose) which is then converted to the osazone. Isbell[42] depicts this reaction in terms



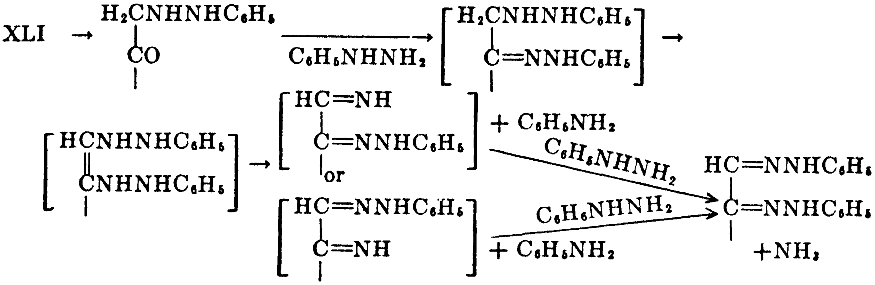
of electron displacement as follows.

[41] F. Weygand, *Ber.*, **73**, 1259 (1940).

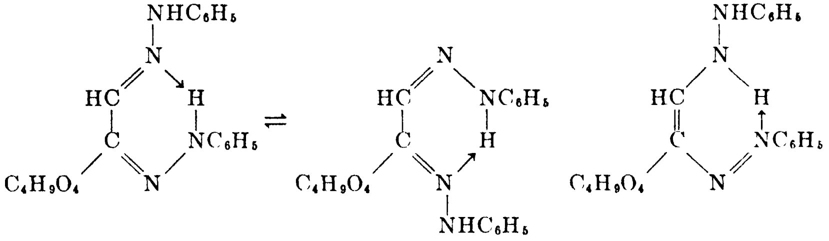
[42] H. S. Isbell, *Ann. Rev. Biochem.*, **12**, 207 (1943).



The second alternative route suggested by Weygand is shown below.



It will be seen that the cleavage of the hypothetical hydrazo derivatives to the hypothetical imino compounds obviates the necessity of assuming direct oxidation by phenylhydrazine. Fieser and Fieser[43] inquire why osazone formation ceases with the introduction of two phenylhydrazine residues, and seek to explain this fact by the postulation of chelate tautomeric ring structures XLIII or to the existence of a resonance



XLIII XLIV

hybrid XLIV. No experimental evidence is available as yet in support of these views, and in the opinion of the writer of this article the facts are explained equally well by the adoption of the keto-pyranose phenylosazone structure which has been discussed earlier. If the oxide ring

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(43) L. F. Fieser and Mary Fieser, "Organic Chemistry," D. C. Heath and Co., Boston, p. 351 (1944).

engages C_2 the $\overset{|}{\underset{|}{C}}{=}NNHC_6H_5$ is converted into the hydrazide residue $-\overset{|}{\underset{|}{C}}-NHNHC_6H_5$ (XII), and no isomerization to an enolic structure involving C_3 is possible since the $>C{=}N-$ grouping has been removed.

An instance of osazone formation in which substitution takes place on the second and third carbon atoms (in 1-desoxy-D-psicose) has been reported.[44] This case is of interest since the possibility arises that with other ketoses, as psicose, 2,3- as well as 1,2-osazones might be produced side by side. The commonest ketose, D-fructose, gives D-glucose phenylosazone with such extreme ease, that it is just possible the phenomenon may have escaped observation.

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(44) M. L. Wolfrom, A. Thompson and E. F. Evans, *J. Am. Chem. Soc.*, **67**, 1793 (1945).

THE CHEMISTRY AND CONFIGURATION OF THE CYCLITOLS

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I. Introduction

The hexahydroxycyclohexanes have long been of particular interest to those concerned with the carbohydrates. Originally this interest arose from the fact that they are isomeric with the hexoses and resemble the sugars in water solubility and sweetness; it has been sustained by their unique properties, their stereochemical relationships and, finally, by the tantalizing question of their significance in nature. The present review will deal chiefly with the chemistry and configurations of the hexahydroxycyclohexanes or inositols, as they are frequently called, and will touch on certain other closely related polyhydroxycarbocyclic substances. The convenient term *cyclitol* has come to denote the type of compound that will be considered herein.

Theory predicts the possibility of the existence of nine stereoisomeric hexahydroxycyclohexanes. No completely satisfactory convention has been devised for depicting these on a plane surface but custom and convenience at the present time justify alteration of the bond angles to depict the inositols by the perspective formulas I to IX.[1]

II. THE INOSITOLS (HEXAHYDROXYCYCLOHEXANES)

1. *meso-Inositol*

The very widespread occurrence of *meso*-inositol (m. p. 225°) in both plant and animal sources makes this substance by far the most extensively investigated of the cylitols; in fact, the comparative rarity of the other inositols has justified the general retention of the name inositol to denote *meso*-inositol although, as pointed out above, the term has been extended to its isomers. *meso*-Inositol and the equally common name, inactive inositol, are obviously poor names for this compound since six of the other inositols are also optically inactive. The sole attempt at a rigorous systematic nomenclature for the cyclitols is that of Maquenne[2] who suggested the use of a fraction to denote the positions of the hydroxyl groups. Thus formula V may be named $\frac{2,4,5,6}{1,3}$-hexahydroxycyclohexane.

meso-Inositol was discovered in 1850 by Scherer[3] in Liebig's meat extract and given the name inositol. In the succeeding years it was found in a great variety of plant and animal sources,[4] both uncombined and in the form of various derivatives. For example, two monomethyl ethers are known—the optically active bornesitol found in Borneo and Madagascar rubber by Girard[5] and in the wash water from a rubber factory by Flint and Tollens,[6] and the optically inactive sequoitol found in the heartwood of the redwood (*Sequoia sempervirens*) by Sherrard and Kurth.[7,7a] An inactive dimethyl ether, dambonitol, has also been isolated from Gabon rubber,[8] the juice of *Castilloa elastica*[9] and from a Sumatran

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(1) The hydrogen atoms have been in the main omitted for clarity throughout this review.

(2) L. Maquenne, "Les Sucres et leurs Principaux Dérivés," Carré et Naud, Paris, p. 14 (1900).

(3) J. Scherer, *Ann.*, **73**, 322 (1850).

(4) See Beilstein, 4th ed., **6**, 1194; Suppl. 1, **6**, 588; Suppl. 2, **6**, 1158.

(5) A. Girard, *Compt. rend.*, **73**, 426 (1871).

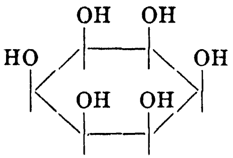
(6) E. R. Flint and B. Tollens, *Ann.*, **272**, 288 (1892).

(7) E. C. Sherrard and E. F. Kurth, *Ind. Eng. Chem.*, **20**, 722 (1928).

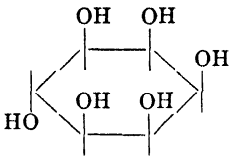
(7a) E. C. Sherrard and E. F. Kurth, *J. Am. Chem. Soc.*, **51**, 3139 (1929).

(8) A. Girard, *Compt. rend.*, **67**, 820 (1868).

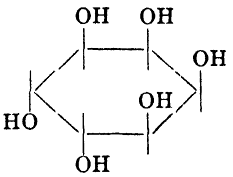
(9) C. O. Weber, *Ber.*, **36**, 3108 (1903).



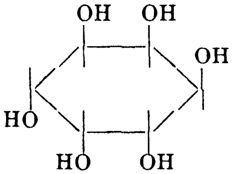
I



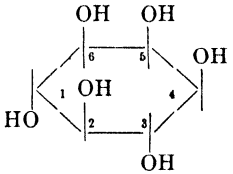
II
epi-Inositol



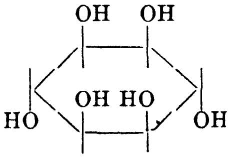
III
allo-Inositol



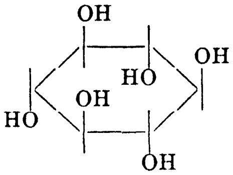
IV



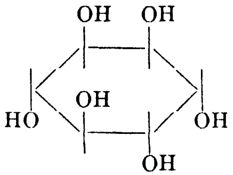
V
meso-Inositol



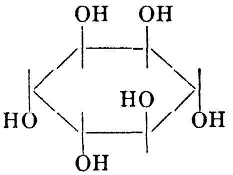
VI
muco-Inositol



VII
Scyllitol



VIII
dextro-Inositol



IX
levo-Inositol

latex.[10,11] Recent work has uncovered a water-soluble, non-dialyzable complex of *meso*-inositol in the liver of the mouse[12] and lipositol, an inositol-containing phospholipid, from the soybean.[13] *meso*-Inositol also occurs widely as the calcium and magnesium salt of its hexaphosphoric acid ester, phytin. The steep liquor from the cornstarch refining process is a convenient source of phytin. Acid hydrolysis or mere autoclaving with water may be employed to free the inositol from this ester on a large scale.[14] The discovery[15] in 1928 that Bios I, a factor essential for the growth of certain yeasts, is identical with *meso*-inositol has stimulated a large amount of research on the biological functions of this cyclitol. The

(10) A. W. K. DeJong, *Rec. trav. chim.*, **27,** 257 (1908).

(11) The structures of these various ethers of *meso*-inositol have not been investigated. However, in the light of the present knowledge of the configuration of *meso*-inositol it is apparent that bornesitol must be 3- or 4-methyl-*meso*-inositol or one of the corresponding enantiomorphs, 1- or 6-methyl-*meso*-inositol. Assuming that sequoitol and dambonitol are not resolvable, the former must be either 2- or 5-methyl-*meso*-inositol while the latter is 1,3-, 2,5- or 4,6-dimethyl-*meso*-inositol.

(12) D. W. Woolley, *J. Biol. Chem.*, **139,** 29 (1941).

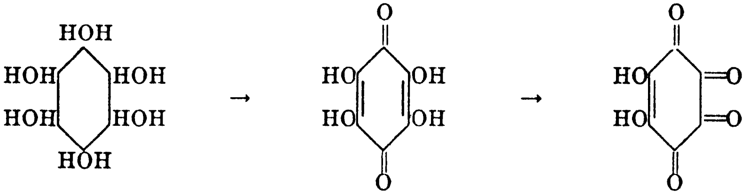
(13) D. W. Woolley, *J. Biol. Chem.*, **147,** 581 (1943).

(14) E. Bartow and W. W. Walker, *Ind. Eng. Chem.*, **30,** 300 (1938); F. A. Hoglan and E. Bartow, *ibid.*, **31,** 749 (1939).

(15) Edna V. Eastcott, *J. Phys. Chem.*, **32,** 1094 (1928).

nutritional significance of *meso*-inositol has recently been reviewed by Woolley.[16]

It was Maquenne[17] who first threw light on the structure of inositol by showing that violent treatment with hydriodic acid gave recognizable quantities of benzene, phenol and triiodophenol.[17a] Oxidation with concentrated nitric acid at 100° gave tetrahydroxy-*p*-benzoquinone and rhodizonic acid, further demonstrating the presence of a six-carbon cycle.[18]



Tetrahydroxy-*p*-benzoquinone Rhodizonic acid

From these facts Maquenne concluded that he was dealing with a hexahydroxycyclohexane. Confirmation of this structure was obtained in 1914 by Wieland and Wishart[19] who found that catalytic reduction of hexahydroxybenzene gave more than a 50% yield of *meso*-inositol.

The difficult proof of the configuration of *meso*-inositol has come only recently from two independent sources, each proof representing the culmination of a long series of researches. The work of T. Posternak, although second in actual priority, will be described first.

Of the nine possible configurations, VIII and IX were eliminated at the outset since they contain no plane of symmetry and represent optically active compounds while *meso*-inositol is inactive and unresolvable. The action of phosphatase on *meso*-inositol hexaphosphoric acid ester[20]

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(16) D. W. Woolley, *J. Nutrition*, **28**, 305 (1944).

(17) L. Maquenne, *Compt. rend.*, **104**, 225, 297 (1887).

(17*a*) For the action of other halogen acids on *meso*-inositol and on some of its isomers see page 57.

(18) The distinctive coloration of the alkaline earth salts of these quinones has long been the basis for a general qualitative test for the inositols; see: J. Scherer, *Ann.*, **81**, 375 (1852); E. Salkowski, *Z. physiol. Chem.*, **69**, 466 (1910).

In recent years the salts of tetrahydroxy-*p*-benzoquinone and rhodizonic acid have found considerable use as indicators in the volumetric determination of sulfates with barium salt solution. In this connection, F. A. Hoglan and E. Bartow, *J. Am. Chem. Soc.*, **62**, 2397 (1940), have studied the nitric acid oxidation of inositol, and P. W. Preisler and L. Berger, *J. Am. Chem. Soc.*, **64**, 67 (1942), have developed methods for preparing pure salts of tetrahydroxy-*p*-benzoquinone and rhodizonic acid from inositol.

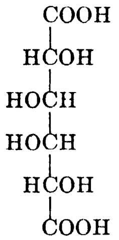
(19) H. Wieland and R. S. Wishart, *Ber.*, **47**, 2082 (1914).

(20) S. Posternak and T. Posternak, *Compt. rend.*, **186**, 261 (1928); *Helv. Chim. Acta*, **12**, 1165 (1929).

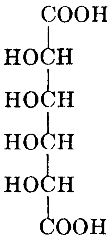
led to the isolation of optically active di- and tetraphosphoric acid esters of *meso*-inositol. This fact eliminated configuration I since all possible di- and tetra- substituted derivatives of this form would possess a plane of symmetry. Further action of phosphatase produced an inactive monophosphoric acid ester. Thus configuration III is eliminated since a resolvable mono derivative of biochemical origin is unlikely. Oxidation of a mixture of the mono- and diphosphoric acid esters of *meso*-inositol with nitric acid gave monophosphoric acid esters of tartaric acid which, upon hydrolysis, yielded *meso* and racemic tartaric acids. If it be assumed that no Walden inversions have taken place, these facts indicate the untenability of configurations I and VII.

Recourse was then had to the direct oxidation of *meso*-inositol with alkaline potassium permanganate, and a hexaric acid[20a] was obtained which proved to be identical with allomucic acid prepared previously by Emil Fischer.[21] Since the configuration assigned to this acid placed the four hydroxyl groups in a *cis-cis-cis* relationship, all the remaining configurations for *meso*-inositol save II are seen to be inapplicable. From the mother liquors of the oxidation mixture D,L-glucosaccharic acid was also isolated. This result, however, only showed that form IV was untenable.

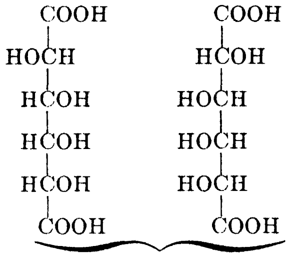
Fortunately, later work by Posternak[22] revealed a flaw in this proof of configuration which partially invalidated the original conclusion and re-opened the matter for investigation. This later work showed that Fischer had, as he suspected,[23] been mistaken as to the configuration of his "allomucic acid." He had treated mucic acid with pyridine at an elevated temperature in the expectation that inversion would take place at both carbons two and five. However, when Posternak prepared



Mucic acid



Allomucic acid



D,L-Talomucic acid

[20a] The term hexaric acid was first proposed by G. Pierce, *J. Biol. Chem.*, **23**, 328 (1915), and refers to the dicarboxylic acids of the aldohexoses.

[21] E. Fischer, *Ber.*, **24**, 2136 (1891).

[22] T. Posternak, *Helv. Chim. Acta*, **18**, 1283 (1935).

[23] E. Fischer, *Ber.*, **27**, 3214, 3220 (1894).

D,L-talomucic acid by an unequivocal series of reactions he found his product to be identical with the "allomucic acid" which he had obtained from *meso*-inositol. From this work it became evident, first, that Fischer had been wrong in his assumption that two inversions had taken place in the pyridine treatment of mucic acid and, second, that the configuration of *meso*-inositol was again in doubt. Configuration IV, it will be remembered, was rendered untenable by the isolation of D,L-glucosaccharic acid. Of the remaining forms (II, V, VI) not excluded by previous evidence only II and V may give rise to D,L-talomucic acid on oxidation. Examination of formulas II and V shows that oxidative cleavage of II could give rise to D,L-talomucic, D,L-glucosaccharic and allomucic acids. Likewise configuration V might give D,L-talomucic, D,L-glucosaccharic and D,L-idosaccharic acids. Therefore the isolation of allomucic acid or D,L-idosaccharic acid was to be desired as these are the sole definitive derivatives. Such was the state of the *meso*-inositol problem from 1935 to 1941.

In 1941 Posternak[24] studied *scyllo-meso*-inosose (see p. 65), a pentahydroxycyclohexanone which had been obtained by Kluyver and Boezaardt[25] from the biochemical oxidation of *meso*-inositol. Cautious oxidation of *scyllo-meso*-inosose with permanganate led Posternak to the isolation of a hexaric acid which he identified as D,L-glucosaccharic acid. Since, of the two forms II and V, only II can give an optically inactive inosose oxidizable to D,L-glucosaccharic acid, this evidence appeared to settle the configuration of *meso*-inositol.

In 1942, however, Mrs. Gerda Dangschat[26] announced the isolation of D,L-idosaccharic acid from the oxidation of a derivative of *meso*-inositol. Several months later Posternak[26a] published proof that his hexaric acid from the oxidation of inosose was actually D,L-idosaccharic acid and not D,L-glucosaccharic acid. Thus, by two separate paths *meso*-inositol was finally proven to be V or $\frac{2,4,5,6}{1,3}$-hexahydroxycyclohexane.

Dangschat's proof of the configuration of *meso*-inositol involved acetonation. The apparently great resistance of *meso*-inositol toward condensation with ketones and aldehydes stands in sharp contrast to the behavior of its acyclic analogs, the sugar alcohols. In addition, the inositols differ markedly from the sugar alcohols in their higher melting points and by their failure to increase the conductivity of boric acid

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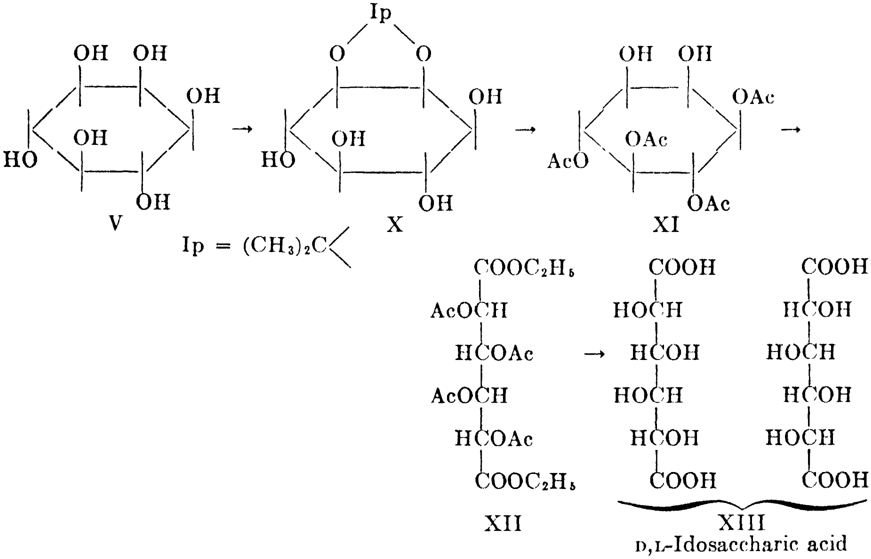
(24) T. Posternak, *Compt. rend. soc. phys. hist. nat. Genève*, **58**, 44 (1941); *C. A.*, **37**, 3063 (1943).

(25) A. J. Kluyver and A. G. J. Boezaardt, *Rec. trav. chim.*, **58**, 956 (1939).

(26) Gerda Dangschat, *Naturwissenschaften*, **30**, 146 (1942).

(26*a*) T. Posternak, *Helv. Chim. Acta*, **25**, 746 (1942).

solutions.[26b,26c] Numerous attempts to prepare cyclic acetals from *meso*-inositol and from the pentahydroxycyclohexane quercitol (see page 66), had hitherto proved unsuccessful.[26b,26d,27] and barred the use of this elegant classical mode of attack on the configuration of *meso*-inositol. Dangschat succeeded in acetonating *meso*-inositol by using as a catalyst a mixture of zinc chloride and acetic acid. According to Meerwein,[28] this mixture forms a so-called "ansolvo acid," the latter being a very effective catalyst for acetonations. Refluxing *meso*-inositol with a large excess of acetone containing 10% zinc chloride and 10% glacial acetic acid gave the 5,6-isopropylidene-*meso*-inositol (X) and an equal amount of its enantiomorph, 4,5-isopropylidene-*meso*-inositol. Acetylation gave a tetraacetate which was distilled and isolated. Removal of the acetone residue by treatment with acid gave the tetraacetate XI which was then oxidized with lead tetraacetate. The resulting amorphous dialdehyde, oxidized with peracetic acid, then esterified with diazoethane, produced XII, a diethyl tetraacetyl-hexarate. This substance was found to be identical with diethyl tetraacetyl-D,L-idosaccharate and affords an unequivocal decision between configurations II and V for *meso*-inositol.



The oxidation of *meso*-inositol by periodic acid, studied by Fleury and

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(26*b*) J. Böeseken and Mlle. A. Julius, *Rec. trav. chim.*, **45**, 489 (1926).

(26*c*) J. C. Krantz, Jr., C. J. Carr and F. F. Beck, *J. Phys. Chem.*, **40**, 927 (1936).

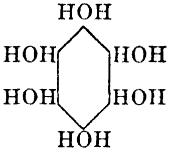
(26*d*) P. Karrer, *Helv. Chim. Acta*, **9**, 116 (1926).

(27) F. Micheel, H. Ruhkopf and F. Suckfüll, *Ber.*, **68**, 1523 (1935).

(28) H. Meerwein, *Ann.*, **455**, 227 (1927).

his coworkers,[28a] has led to some rather surprising results. By analogy with the behavior of the acyclic polyols, inositol would be expected to consume six moles of periodic acid with the formation of an equal number of moles of formic acid. Actually, less than the theoretical quantity of formic acid was found and it was observed that carbon dioxide was evolved during the course of the reaction. Experiments showed that *meso*-inositol reduced four moles of periodic acid in the course of a few minutes. The rate of consumption of oxidant then diminished sharply and finally attained a value of 6.7 moles after two or three days. During the course of the reaction the solution showed a reducing value which reached a maximum when two moles of oxidant had been consumed and then disappeared after four moles of periodic acid had been reduced. Titration showed the presence of four moles of acid when four moles of oxidant had been consumed, and the acid titer remained constant thereafter. The evolution of carbon dioxide was found to take place after the rapid initial consumption of four moles of oxidant. Finally, glycolic and formic acids were isolated from the reaction mixture. Pure glycolic acid was found to react with periodic acid to give carbon dioxide and formic acid although, even in the presence of an excess of the oxidant, the reaction failed, for reasons yet unknown, to go to completion.

In explanation of this array of facts Fleury, Poirot and Fievet[28a] have offered the following mechanism. It is suggested that the *meso*-inositol molecule is initially attacked at two points with the formation of two moles of tartronic dialdehyde. This dialdehyde then rapidly rearranges



$$+ 2HIO_4 \rightarrow 2CHO\text{—}CHOH\text{—}CHO + 2H_2O + 2HIO_3$$

to hydroxypyruvic aldehyde and is cleaved to glycolic and formic acids.

$$2CHO\text{—}CHOH\text{—}CHO \rightarrow 2CH_2OH\text{—}CO\text{—}CHO$$

$$2CH_2OH\text{—}CO\text{—}CHO + 2HIO_4 + H_2O \rightarrow 2CH_2OH\text{—}COOH + 2HCO_2H + 2HIO_3$$

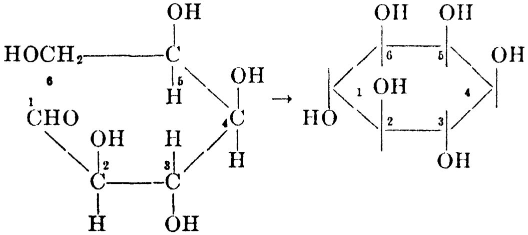
These reactions account for the rapid initial consumption of four moles of periodic acid. The subsequent slow reaction is assumed to involve the incomplete oxidation of glycolic acid.

$$2CH_2OH\text{—}COOH + 4HIO_4 \rightarrow 2HCO_2H + 2CO_2 + 2H_2O + 4HIO_3$$

This unexpected behavior of *meso*-inositol with periodic acid serves to emphasize further the dissimilarity of this compound to its acyclic analogs. It is to be hoped that the action of periodic acid on the other inositols will be similarly studied.

[28a] P. Fleury, G. Poirot and Mme. J. Fievet, *Compt. rend.*, **220**, 664 (1945); see also P. Fleury and Mlle. M. Joly, *J. pharm. chim.*, **26**, 341, 397 (1937); P. Fleury and J. Lange, *ibid.*, **17**, 313 (1933).

The possible role of *meso*-inositol and other inositols in nature has been the cause of much speculation.[29] Of particular significance is the recent interesting discovery of a 1,3-diguanidino-2,4,5,6-tetrahydroxycyclohexane as an integral part of the streptomycin molecule (see page 346). Even before the configuration of *meso*-inositol was known it seemed most likely that it arose in nature by an aldol-type of reaction between carbons one and six of an aldohexose or some derivative thereof. With the discovery that *meso*-inositol is configuratively related to D-glucose this hypothesis becomes more attractive. It will be noted that such a ring closure involves the formation of two new asymmetric centers (1 and 6). Previous work has shown that under such circumstances it is very likely that the configurations of the two new asymmetric carbons will be opposite, as is indeed the case here. Fischer and Baer,[30] for instance, found that the condensation of D-glyceraldehyde and dihydroxyacetone produced D-fructose and D-sorbose while neither D-psicose nor D-tagatose, in which carbons three and four have the same configuration, could be detected.



V

D-Glucose *meso*-Inositol

However, despite many attempts,[31] it is only within the last few years that progress has been made toward the successful cyclization of hexose derivatives to inositol derivatives. Fernández, Izquierdo and Martínez[31a] have reported the presence of a cyclase in the leaves of *Lactuca virosa* L. which converts D-glucose to a material giving the Scherer test for inositol. Since this color test is general for the inositols a definite statement regarding the nature of the product will have to await its isolation and identification.

Grosheintz and Fischer[32] have sought to activate the hydrogen atoms

(29) See H. O. L. Fischer, *Harvey Lectures*, Ser. **40,** 156 (1945).

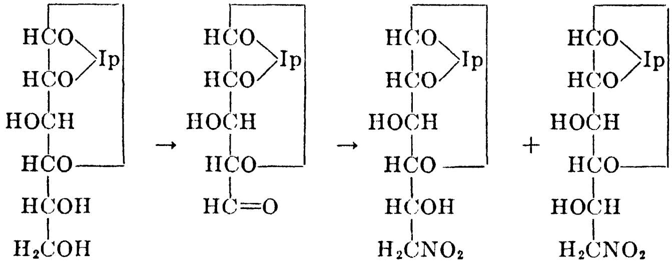
(30) H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **19,** 519 (1936).

(31) See F. Micheel, *Ann.*, **496,** 77 (1932), and the references therein cited.

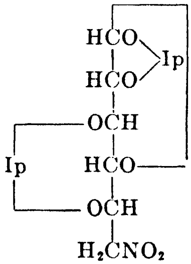
(31*a*) O. Fernández, G. Izquierdo and E. Martínez, *Farm. nueva* (Madrid), **9,** 563 (1944); *C. A.*, **40,** 4115 (1946).

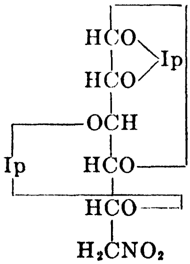
(32) J. M. Grosheintz and H. O. L. Fischer, *J. Am. Chem. Soc.*, *in press* (1948). See also ref. 29.

attached to carbon six of D-glucose by replacing the hydroxyl group on carbon six with a nitro group. Their synthetic course started with 1,2-isopropylidene-D-glucofuranose (XIV) which was cleaved with lead tetraacetate to give 1,2-isopropylidene-D-*xylofurano*-trihydroxy-glutaric dialdehyde (XV). When combined with nitromethane in an alkaline medium this aldehyde gave the two expected diastereoisomers, 1,2-isopropylidene-6-nitro-6-desoxy-D-glucofuranose (XVI) and the corresponding derivative of L-idose (XVII). These isomers were separated by the rather novel process of selective acetonation. The L-idose derivative, having the free hydroxyl groups on carbon atoms three and five in the *cis* position, could be acetonated easily to 1,2:3,5-diisopropylidene-6-nitro-6-desoxy-L-idofuranose (XVIII) while the third and fifth hydroxyls in the D-glucose derivative were not suitably situated to form, 1,2:3,5-diisopropylidene-6-nitro-6-desoxy-D-glucofuranose (XIX). An acetone solution of hydrochloric acid was found to dissolve the L-idose derivative but not the D-glucose derivative. Subsequent removal of the acetone residues furnished 6-nitro-6-desoxy-L-idose (XXI) and 6-nitro-6-desoxy-D-glucose (XX). Both of these diastereoisomers were found to condense in slightly alkaline solution to a mixture of only two mononitromonodesoxyinositols, the same two compounds rather surprisingly arising from either the D-glucose or the L-idose derivative. In explanation of

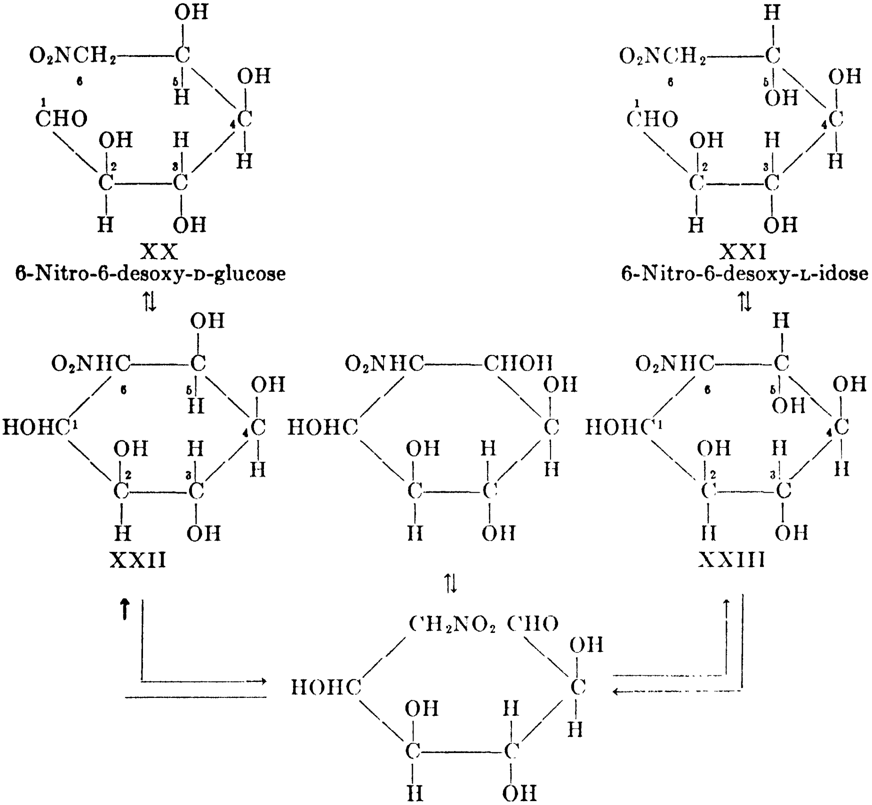

XIV XV XVI XVII

1,2-Isopropylidene-D-glucofuranose


XVIII


XIX

this the authors suggest that an equilibrium may be formed and that the initial products XXII and XXIII may reopen between carbon atoms five and six as well as between one and six. The configurations of carbons two, three and four in the products were shown to be the same as the corresponding ones in the starting materials. The configuration of the remaining carbon atoms is still in doubt. Evidence of the presence of a carbocyclic ring was obtained when acetylation with acetic anhydride and pyridine gave 5-nitro-resorcinol diacetate[33] in almost quantitative yield. While it is apparent that this particular mode of cyclization of hexose derivatives can hardly be called an imitation of a natural process, it does for the first time demonstrate that such a ring closure is feasible and renders most improbable the suggestion[34] that intramolecular forces hold the hexoses in a form such that the approach of carbon one to carbon six is very difficult.



XX
6-Nitro-6-desoxy-D-glucose

XXI
6-Nitro-6-desoxy-L-idose

XXII

XXIII

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(33) Compare with the similar aromatization of inosose, page 64.

(34) F. Micheel, *Ann.*, **496**, 77 (1932).

2. *dextro-Inositol and levo-Inositol*[35]

Dextrorotatory inositol occurs in various plants as its monomethyl ether, pinitol, which was first mentioned by Berthelot[36] as a constituent of the exudate of *Pinus lambertiana* Dougl. More recently it has been discovered in the heartwood of the redwood (*Sequoia sempervirens*)[7] and in two varieties of the loco weed, *Astragalus earlei and Oxytropis lambertii.*[37]

Levorotatory inositol also occurs as a monomethyl ether, first discovered by Tanret[38] in the bark of the quebracho tree and named by him quebrachitol. Subsequent researches have encountered this ether in *Hakea laurina* R. Br.,[39] in the stems of *Haplophyton cimicidum,*[40] in *Acalypha indica,*[41] in the "red oil" from Minnesota wild hemp[42] and in the maples *Acer pseudoplatanus* and *A. platanoides* L.[43] Plouvier[43a] has reported the occurrence of quebrachitol in numerous Aceraceae and Sapindaceae. The latex of the rubber tree, *Hevea brasiliensis,* however, is the most convenient source of quebrachitol.[44] Since relatively large quantities of the substance are normally discarded with the serum after precipitation of the rubber, the commercial exploitation of quebrachitol has received consideration.[45] The sweetness of quebrachitol, which is considerably less than that of sucrose or D-glucose, has led to its investigation as a sweetening agent for diabetics. The untoward effects incident upon the ingestion of considerable quantities of quebrachitol were found, however, to make quebrachitol unsuitable for this purpose.[46]

The positions of the methyl groups in pinitol and quebrachitol have not been ascertained. The physical properties of the two substances

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(35) The prefixes *dextro-* and *levo-* are synonymous with dextrorotatory and levorotatory and refer to rotation only.

(36) M. Berthelot, *Compt. rend.*, **41,** 392 (1855).

(37) D. C. Pease, M. J. Reider and R. C. Elderfield, *J. Org. Chem.*, **5,** 198 (1940).

(38) C. Tanret, *Compt. rend.*, **109,** 908 (1889).

(39) E. Bourquelot and H. Hérissey, *Compt. rend.*, **168,** 414 (1919).

(40) E. P. Clark, *J. Am. Chem. Soc.*, **58,** 1009 (1936).

(41) C. Rimington and G. C. S. Roets, *Onderstepoort J. Vet. Sci. Animal Ind.*, **9,** 193 (1937); *C. A.*, **32,** 4629 (1938).

(42) R. Adams, D. C. Pease and J. H. Clark, *J. Am. Chem. Soc.*, **62,** 2194 (1940).

(43) M. Malmy and P. Bouvet, *J. pharm. chim.*, **2,** 5 (1942).

(43*a*) V. Plouvier, *Compt. rend.*, **224,** 1842 (1947).

(44) A. W. K. DeJong, *Rec. trav. chim.*, **25,** 48 (1906); A. Contardi, *Ann. chim. applicata,* **14,** 281 (1924); T. G. Levi, *Rubber Chem. Tech.*, **3,** 1 (1930); T. Posternak, *Helv. Chim. Acta,* **19,** 1007 (1936); J. McGavack and G. B. Binmore, U. S. Pat. 1,758,616 (1930); W. J. Hart, U. S. Pat. 2,378,141 (1945).

(45) E. Rhodes and J. L. Wiltshire, *J. Rubber Research Inst. Malaya,* **3,** 160 (1932); *C. A.*, **26,** 4502 (1932).

(46) R. A. McCance and R. D. Lawrence, *Biochem. J.*, **27,** 986 (1933).

indicate that these ethers are not enantiomorphs; hence the methyl groups are not in corresponding positions. Treatment of either pinitol or quebrachitol with hot concentrated hydriodic acid removes the methyl group and gives nearly quantitative yields of the corresponding cyclitol.

As mentioned above, there can be but two optically active inositols, VIII and IX. It is impossible to decide *a priori* which of these configurations corresponds to *dextro-* and which to *levo*-inositol. The identity of *levo*-inositol with configuration IX was demonstrated by Posternak[47] in 1936. Examination of the formulas will show that mild permanganate oxidation of VIII might be expected to afford L-glucosaccharic, D-mannosaccharic, L-idosaccharic and mucic acids. Its isomer IX would yield mucic acid, D-glucosaccharic, L-mannosaccharic and D-idosaccharic acids. Posternak oxidized *levo*-inositol with potassium permanganate and was able to isolate mucic and D-glucosaccharic acids; levorotatory inositol therefore corresponds to IX and its enantiomorph to formula VIII.

Although pinitol and quebrachitol are resistant to the action of *Acetobacter suboxydans,* Chargaff and Magasanik[47a] have reported that *levo*-inositol is oxidized by this organism to a tetrahydroxycyclohexanedione. Since the phenylosazone from this compound consumes three moles of periodic acid, the authors concluded that the carbonyl groups were adjacent. A similar compound was obtained from *dextro*-inositol although in smaller yield.

Racemic inositol has been reported as a constituent of fresh mistletoe berries where it occurs accompanied by *meso*-inositol[48] and also in small quantity in the stems of the liana "Efiri."[49] Historically, racemic inositol shares with Fischer's D,L-mannonic acid lactone the distinction of being the first racemate produced in the sugar series.[50,51]

The replacement of the hydroxyl groups of *meso*-inositol and its isomers by halogen atoms has been the object of considerable study. The phosphorus halides and hydrogen iodide (see p. 48) cause extensive decomposition with the formation of aromatic halogen derivatives. Müller[52] investigated the action of various halogen acids in acetic acid solution on *meso*-inositol and scyllitol (see page 60) and obtained the same products from both cyclitols. In addition to a variety of mono- and dihalogenated desoxycyclitols, whose structures still remain in doubt, Müller obtained two apparently new cyclitols which he named *iso-* and

(47) T. Posternak, *Helv. Chim. Acta,* **19,** 1007 (1936).
(47a) E. Chargaff and B. Magasanik, *J. Biol. Chem.,* **165,** 379 (1946).
(48) G. Tanret, *Compt. rend.,* **145,** 1196 (1907).
(49) E. Castagne, *Congo,* **II,** 357 (1934); *C. A.,* **30,** 4539 (1936).
(50) L. Maquenne and C. Tanret, *Compt. rend.,* **110,** 86 (1890).
(51) E. Fischer, *Ber.,* **23,** 370 (1890).
(52) H. Müller, *J. Chem. Soc.,* **91,** 1780 (1907); *ibid.,* **101,** 2383 (1912).

pseudo-inositol. Evidently these arose from the inversion of the configuration of one or more carbon atoms in *meso*-inositol and scyllitol. Although it is to be presumed that the inversion is accomplished through the formation and subsequent hydrolysis of a halogen compound, no such intermediate was isolated. *iso*-Inositol was found to melt at 244° and to afford a hexaacetate melting at 112° and a benzoate melting at 213°. Since racemic inositol melts at 253° and has a hexaacetate melting at 111° and a benzoate at 217°,[48,50] it is to be suspected that *iso*-inositol is actually racemic inositol. This suspicion is enhanced by the similarity between the crystallographic constants for racemic inositol[52a] and those reported by Müller for *iso*-inositol. The *pseudo*-inositol which Müller obtained in very small quantity gave amorphous products both when acetylated and when benzoylated. No further notice regarding these inositols has appeared in the literature.

Griffin and Nelson[52b] repeated, in part, the work of Müller and made the further observation that acetyl chloride and acetyl bromide react with *meso*-inositol much as did Müller's glacial acetic acid solution of the hydrogen halides. It may be noted that apparently the same dibromodidesoxyinositol, melting at 216°, was obtained from *meso*-inositol and scyllitol (as the tetraacetate by Müller) and from pinitol (monomethyl-*dextro*-inositol).

Majima and Simanuki[53] found that *meso*-inositol reacts with thionyl chloride only in the presence of pyridine. Among the products isolated was a monochloro-desoxy-inositol, a tetrachloro-tetradesoxy-inositol, a tri- and a tetrachlorobenzene and a tri- and a tetrachlorophenol.

Apparently complete replacement of the hydroxyl groups in *meso*-inositol by halogen has never been accomplished. The reverse procedure, hydrolysis of the benzene hexahalides, has likewise failed although Milhorat and Barthels[54] have claimed the synthesis of an α-tocopheryl ether of a cyclitol by heating α-tocopherol with benzene hexachloride in absolute alcoholic potassium hydroxide under an atmosphere of nitrogen.

3. *allo-Inositol and muco-Inositol*

Dangschat and Fischer[55] have most elegantly extended the chemistry of the cyclitols by an investigation of the naturally occurring cyclitol, conduritol (XXIV). This optically inactive substance was discovered in

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(52*a*) G. Wyrouboff, *Bull. soc. franc. minéral.*, **25**, 165 (1902); *Chem. Zentr.*, **II**, 1498 (1902).

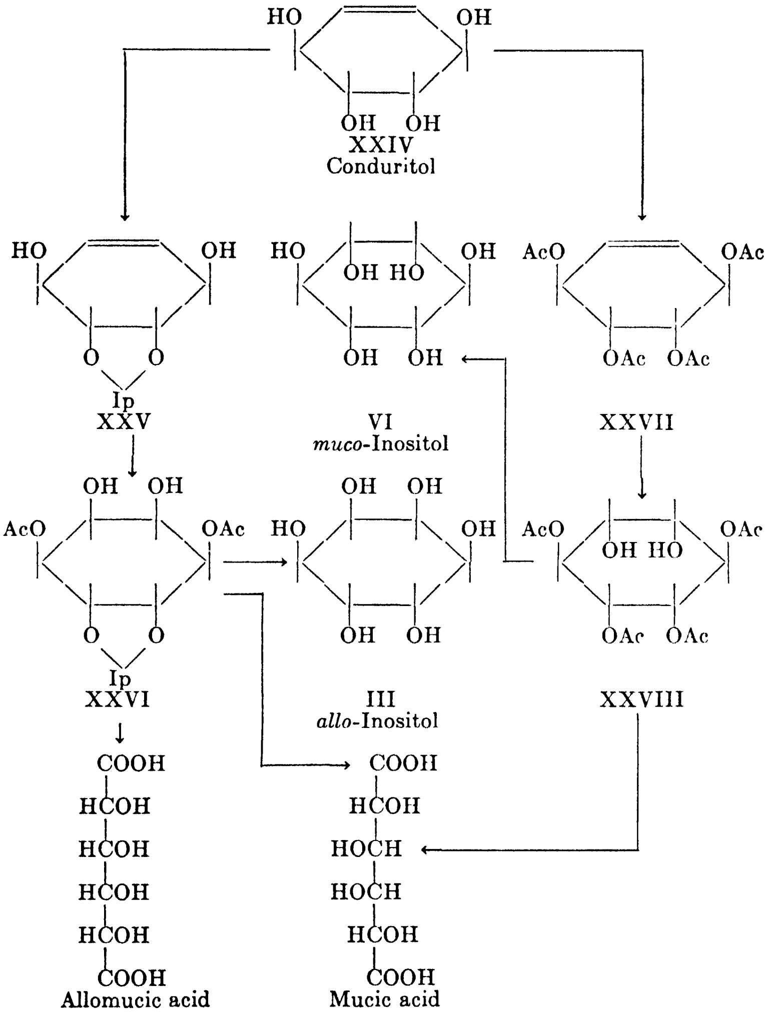
(52*b*) E. G. Griffin and J. M. Nelson, *J. Am. Chem. Soc.*, **37**, 1552 (1915).

(53) R. Majima and H. Simanuki, *Proc. Imp. Acad.* (Tokyo), **2**, 544 (1927).

(54) A. T. Milhorat and W. E. Barthels, *Science*, **101**, 93 (1945).

(55) Gerda Dangschat and H. O. L. Fischer, *Naturwissenschaften*, **27**, 756 (1939).

1908 by Kubler[56] in the bark of the condurango tree (*Marsdenia condurango*) and was shown by its analysis and reactions to be a tetrahydroxycyclohexene. Dangschat and Fischer succeeded in acetonating it to obtain a monoacetone derivative which, being stable to the action of



lead tetraacetate, must have structure XXV or a similar one having both the free hydroxyl groups below the plane of the ring. Acetylation of these two remaining free hydroxyls followed by hydroxylation of the double bond gave XXVI and this yielded mucic acid on successive oxida-

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(56) K. Kubler, *Arch. Pharm.*, **246**, 620 (1908).

tion with lead tetraacetate and perpropionic acid with subsequent hydrolysis. The configuration of conduritol is therefore correctly represented by XXIV, a fact substantiated by Kern, Fricke and Steger[57] who were able to obtain mucic acid from conduritol by direct oxidation. These facts, however, fail to establish the configuration of the intermediate XXVI since the positions of the two hydroxyl groups with respect to the plane of the ring are uncertain. Acetylation was employed to protect these hydroxyls; the acetone group was then removed and stepwise oxidation and hydrolysis were employed to yield a hexaric acid, recognized as allomucic acid. The configuration of XXVI was established as that of a new inositol, named *allo-inositol* (III) which was obtained from XXVI by alternate acid and alkaline hydrolysis.

Two more inositols are theoretically possible products from the hydroxylation of the double bond of conduritol or its derivatives, and Dangschat and Fischer were able to obtain one of these. Hydroxylation of tetraacetylconduritol (XXVII) produced a tetraacetylinositol (XXVIII) which, like XXVI, gave mucic acid on oxidation and hydrolysis. Alkaline hydrolysis of XXVIII gave *muco-inositol* (VI), so named because it may be split in either of two ways to give mucic acid. It may be noted that, had the hydroxyl groups entering the double bond of the tetraacetate XXVII taken a *trans* position with respect to each other, the final product would have been racemic inositol.

4. *Scyllitol*

Another naturally occurring inositol, scyllitol, was discovered by Staedeler and Frerichs[58] in the organs of various plagiostomous fish. Vincent and Delachanal[59] in 1887 isolated an inositol from acorns which they named *quercinitol* while Müller[60] in 1907 reported the presence of a similar compound, which he named *cocositol,* in the leaves of *Cocos plumosa* and *Cocos nucifera.* Later work by Müller[52] demonstrated the identity of quercinitol and cocositol with scyllitol. Others have found the compound in the muscle[61] and liver[62] of the spur dogfish (*Acanthia vulgaris*) as well as in the flowers and bracts of the flowering dogwood, *Cornus florida.*[63]

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(57) W. Kern, W. Fricke and H. Steger, *Arch. Pharm.*, **278,** 145 (1940).

(58) G. Staedeler and F. T. Frerichs, *J. prakt. Chem.*, **73,** 48 (1858).

(59) C. Vincent and Delachanal, *Compt. rend.*, **104,** 1855 (1887).

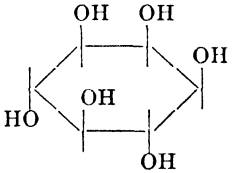
(60) H. Müller, *J. Chem. Soc.*, **91,** 1767 (1907).

(61) M. Mohr, *Z. Biol.*, **98,** 276 (1937).

(62) D. Ackermann and M. Mohr, *Z. Biol.*, **98,** 37 (1937).

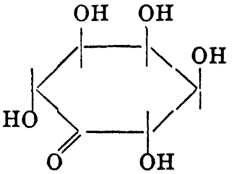
(63) C. E. Sando, K. S. Markley and M. B. Matlack, *J. Biol. Chem.*, **114,** 39 (1936).

The configuration of scyllitol was established by Posternak[24,26a,64] in the course of his work on the configuration of *meso*-inositol. As mentioned previously, the biochemical oxidation of *meso*-inositol gave rise to an optically inactive pentahydroxycyclohexanone. Since there are but two such optically inactive structures (XXIX and XXX) possible from *meso*-inositol, and since further oxidation with permanganate gave D,L-idosaccharic acid, formula XXX may definitely be ascribed to this cyclo-ketose. Reduction with sodium amalgam in dilute acetic acid solution gave two inositols: *meso*-inositol and a cyclitol of higher melting point which proved to be identical with natural scyllitol. Scyllitol must therefore be represented by formula VII, the inositol whose hydroxyl groups all stand in the *trans* relationship.

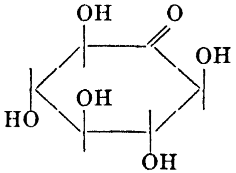


V

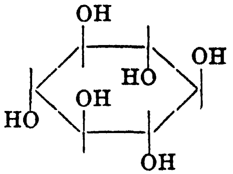
meso-Inositol



XXIX



XXX

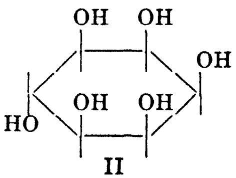


VII

Scyllitol

5. *epi-Inositol*

Cautious nitric acid oxidation of *meso*-inositol afforded Posternak[65] a pentahydroxycyclohexanone. Reduction of this compound or its pentaacetate gave a new inositol which Posternak named *epi*-inositol. Proof of the structure of the pentahydroxycyclohexanone (*epi-meso*-inosose, see the following section) as a racemic mixture of 1-keto- and 3-keto-*meso*-inositol (XXXIII and XXXIV) demonstrated that *epi*-inositol corresponds to formula II.



II

epi-Inositol

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(64) T. Posternak, *Helv. Chim. Acta*, **24**, 1045 (1941).

(65) T. Posternak, *Helv. Chim. Acta*, **19**, 1333 (1936).

III. THE INOSOSES (PENTAHYDROXYCYCLOHEXANONES)

1. *epi-meso-Inosose*[66]

As mentioned earlier, the work of Maquenne published in 1887 demonstrated that *meso*-inositol could be oxidized to a mixture of hydroxy ketones. It remained for Posternak[65] in 1936 to ameliorate the conditions of this nitric acid oxidation so that the primary oxidation product, a penta-hydroxycyclohexanone, could be isolated. This *inosose,* as he named it, proved to bear somewhat the same relationship to the reducing sugars as the cyclitols do to the sugar alcohols. However, in some respects its reactivity surpassed that of the normal ketoses. It rapidly reduced Fehling solution in the cold and consumed about two atoms of iodine when titrated by the method of Willstätter and Schudel[67]—behavior characteristic of an aldose rather than a ketose. With semicarbazide this ketose gave a semicarbazone; with phenylhydrazine, a phenylhydrazone; however, attempts to prepare a phenylosazone failed.[67a] Acylation with acidic catalysts such as zinc chloride or sulfuric acid gave a pentaacetate and a pentabenzoate. Catalytic reduction of the inosose, as well as of its pentaacetate, gave a new inositol which, since it differed from *meso*-inositol only in the configuration of one carbon, was named *epi*-inositol. Reduction of inosose with sodium amalgam in acid medium, on the other hand, gave a mixture of *meso*-inositol and *epi*-inositol. To distinguish this inosose from an isomer later discovered and to show its genetic relationships, Posternak termed it *epi-meso*-inosose.

Although a bicyclic hemiacetal form such as XXXII is sterically possible, Posternak[68] considered, in view of its unusual reducing power, ready reduction and ultraviolet absorption characteristics, that the keto form XXXI is more probable.

The question of the configuration of *epi-meso*-inosose has recently been answered by Posternak.[68] Examination of the formula for *meso*-inositol (V) will reveal that oxidation might give rise to six inososes. Attack at the equivalent positions one and three would give the enantiomorphous pair of inososes (XXXIII and XXXIV). In precisely the same manner, oxidation of carbons four and six would give the enantiomorphous pair XXXV and XXXVI. On the other hand, oxidation at either position two or five would result in the formation of the sym-

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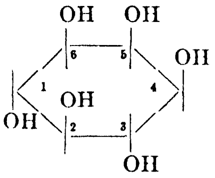
(66) A system of nomenclature for individual pentahydroxycyclohexanones, employing prefixes to denote the generic relationships with the inositols, was suggested by Posternak, ref. 26*a*, and is employed here.

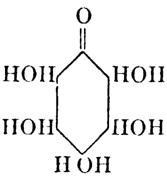
(67) R. Willstätter and G. Schudel, *Ber.*, **51,** 780 (1918).

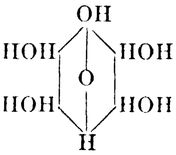
(67*a*) 2-Hydroxy-1-cyclohexanone, on the other hand, gives a phenylosazone; see M. Bergmann and M. Gierth, *Ann.*, **448,** 48 (1926).

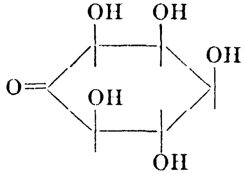
(68) T. Posternak, *Helv. Chim. Acta,* **29,** 1991 (1946).

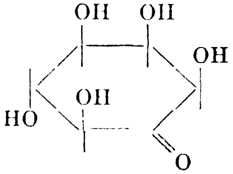
metrical inososes XXIX and XXX. One of these (XXX) represents *scyllo-meso*-inosose, obtained from *meso*-inositol by biochemical oxidation (see page 65). A decision was made among the remaining forms by controlled oxidation with permanganate which gave two hexaric acids:


V
meso-Inositol

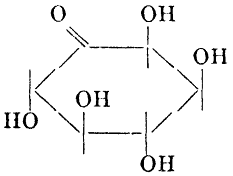

XXXI

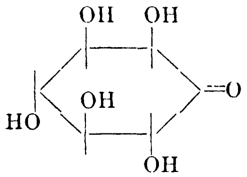

XXXII

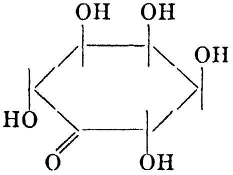

XXXIII

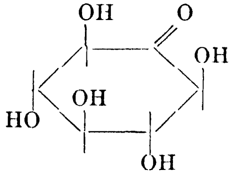

XXXIV

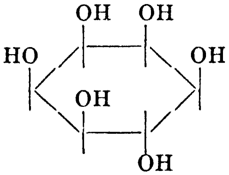
Racemic *epi-meso*-inosose

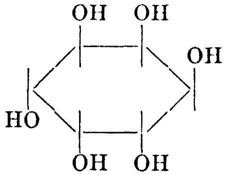

XXXV


XXXVI


XXIX


XXX
scyllo-meso-Inosose

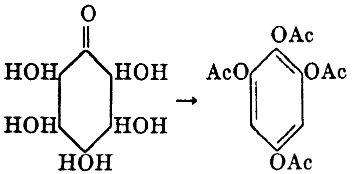

II


IV

D,L-talomucic and D,L-glucosaccharic acids. *epi-meso*-Inosose is therefore the racemate represented by XXXIII and XXXIV while the reduction product *epi*-inositol is II, representing as it does, an inversion of the configuration of carbon one (or the equivalent carbon three) in *meso*-inositol.

Both Chargaff and Magasanik[47a] as well as Posternak[68] have shown that *epi*-inositol is oxidized by *Acetobacter suboxydans* to a levorotatory inosose. Posternak found that reduction of this compound with sodium amalgam in acid solution gives a mixture of *meso*-inositol and *epi*-inositol, proving that the substance is one of the enantiomorphs XXXIII or XXXIV. Since oxidation with permanganate afforded D-talomucic and D-glucosaccharic acids, the levorotatory *epi-meso*-inosose must be represented by XXXIII.

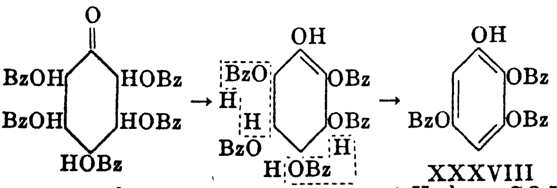
As mentioned earlier, *meso*-inositol, and indeed all the inositols, are readily converted to aromatic compounds by oxidation. Posternak[65] found that inosose has the tendency to form phenolic compounds under very mild conditions. On attempted acetylation of inosose with acetic anhydride in the presence of either pyridine or sodium acetate the expected pentaacetate was not obtained; the product was 1,2,3,5-tetraacetoxybenzene (XXXVII). Similarly, the treatment of inosose pentaacetate with boiling acetic anhydride and pyridine or sodium acetate gave the same product. Inosose pentabenzoate was prepared by the use of benzoyl chloride and zinc chloride. Under the influence of pyridine or sodium acetate in acetic acid the pentabenzoate lost two molecules of benzoic acid to give 1-hydroxy-2,3,5-tribenzoxybenzene (XXXVIII).



Inosose XXXVII

1,2,3,5-Tetraacetoxybenzene

Posternak suggested the following mechanism and pointed out that 1,2,3,5-tetrahydroxybenzene occurs in nature in the form of various methylated derivatives such as iretol, the 2-methyl ether, and antiarol, the 1,2,3-trimethyl ether.

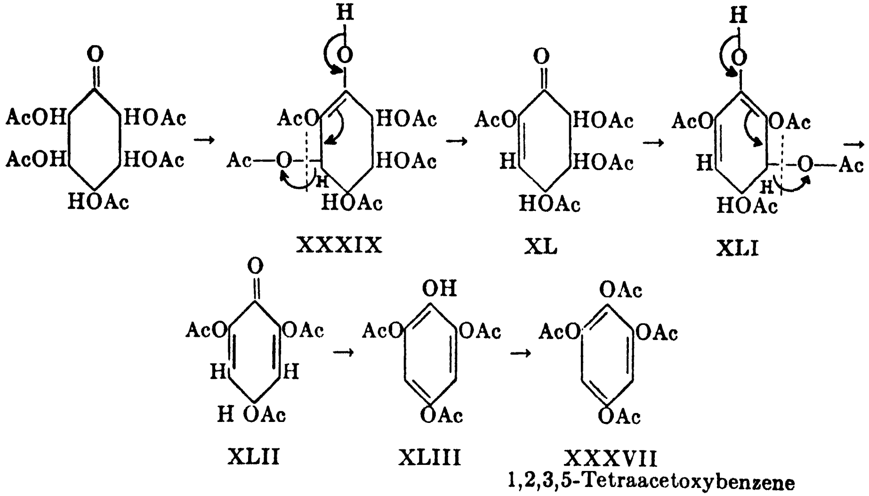


Inosose pentabenzoate

XXXVIII

1-Hydroxy-2,3,5-tribenzoxybenzene

Recently Isbell[69] has proposed an electron displacement mechanism for the aromatization of inosose esters. This attractive interpretation involves the intermediates XXXIX to XLIII. Under the acetylating conditions employed for inosose pentaacetate, XLIII would be converted to 1,2,3,5-tetraacetoxybenzene (XXXVII) and isolated as such. However, the pentabenzoate of inosose was aromatized under non-acetylating conditions and by this mechanism 2-hydroxy-1,3,5-tribenzoxybenzene would be expected. As Isbell pointed out, Posternak's product was not rigorously identified as 1-hydroxy-2,3,5-tribenzoxybenzene (XXXVIII).



2. *scyllo-meso-Inosose*

In 1939, Kluyver and Boezaardt[25] observed that a particular strain of *Acetobacter suboxydans* (Kluyver et de Leeuw) was capable of oxidizing *meso*-inositol[70] in 90% yield to an inosose which had the same melting point as the *epi-meso*-inosose made chemically by Posternak in 1936. Supplied with a culture of the microorganism, Posternak[64] confirmed this observation and showed that, while the optically inactive product of biochemical oxidation resembled very closely that obtained by nitric acid oxidation, the derivatives such as the acetate and benzoate serve to distinguish the two substances. With alkaline acylating reagents this inosose yielded tetrahydroxybenzene derivatives, as had *epi-meso*-inosose.

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[69] H. S. Isbell, *Ann. Rev. Biochem.*, **12**, 213 (1943).

[70] With another strain of the organism, oxidation to a tetrahydroxycyclohexanedione appears possible; see: J. W. Dunning, *Iowa State Coll. J. Sci.*, **14**, 24 (1939); J. W. Dunning, E. I. Fulmer and L. A. Underkofler, *ibid.*, **15**, 39 (1940); W. H. Pitcher, *ibid.*, **16**, 120 (1941).

On oxidation with permanganate[26a] it gave D,L-idosaccharic acid, establishing the structure as XXX. On reduction with sodium amalgam in acetic acid a mixture consisting of *meso*-inositol and what was later proven to be scyllitol was obtained. Following Posternak's suggestion, this ketose is therefore called *scyllo-meso*-inosose. A rigorous name for it may be coined by an adaptation of Maquenne's system of nomenclature for the cyclitols,[2,26a] namely, $\frac{2,4,6}{3,5}$ -pentahydroxy-1-cyclohexanone.

Sprinson and Chargaff[71] have reported that *scyllo-meso*-inosose in bicarbonate solution rapidly reduced six moles of periodic acid while in weakly acid solution the reaction was less complete.

In passing, it may be noted that *Acetobacter suboxydans* attacks the center of a group of three adjacent *cis* hydroxyl groups in both *meso*-inositol and *epi*-inositol.

IV. THE QUERCITOLS (PENTAHYDROXYCYCLOHEXANES)

1. *dextro-Quercitol*

In 1849 Braconnot[72] found a beautifully crystalline substance in the acorn which he mistook for lactose. Two years later, Dessaignes[73] pointed out that this compound was quite distinct from lactose in its properties and represented a new substance which he named *quercite*[74] since it was a product of the oak tree (*Quercus*). Later researches have encountered the substance in a variety of vegetable materials[75] although the acorn is still apparently the best source for preparative purposes. The structure of quercitol as a pentahydroxycyclohexane was early recognized[76] but the much more difficult question of configuration remained open until finally resolved by Posternak[77] in 1932.

Stereochemical theory predicts the possibility of the existence of sixteen pentahydroxycyclohexanes. Of these, four have a plane of symmetry while the remaining twelve represent six enantiomorphous pairs. Since quercitol is optically active, only the twelve active isomers need be considered. Kiliani and Scheibler[78] in 1889 showed that oxidation with nitric acid gave mucic acid—an observation which, as Karrer[79] much later pointed out, further limits the possible configurations. Only four

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(71) D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, **164**, 433 (1946).

(72) H. Braconnot, *Ann. chim. phys.*, [3] **27**, 392 (1849).

(73) M. Dessaignes, *Compt. rend.*, **33**, 308 (1851).

(74) M. Dessaignes, *Compt. rend.*, **33**, 462 (1851).

(75) Beilstein, 4th ed., **6**, 1186; Suppl. 2, **6**, 1151.

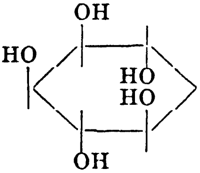
(76) J. Kanonnikoff, *J. prakt. Chem.*, **32**, 497 (1885).

(77) T. Posternak, *Helv. Chim. Acta*, **15**, 948 (1932).

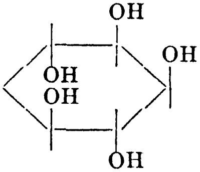
(78) H. Kiliani and C. Scheibler, *Ber.*, **22**, 517 (1889).

(79) P. Karrer, *Helv. Chim. Acta*, **9**, 116 (1926).

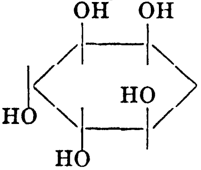
cyclitols, the two racemic pairs XLIV–XLV and XLVI–XLVII, conform to these requirements.



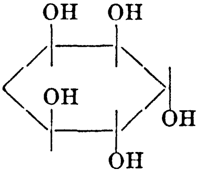
XLIV
dextro-Quercitol



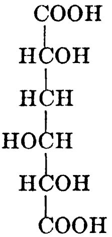
XLV



XLVI



XLVII



XLVIII
Metasaccharinic acid

Posternak employed permanganate oxidation of quercitol and obtained the conclusive derivative, metasaccharinic acid, which from data already published, he showed to have the configuration XLVIII. From this it may be seen that formula XLIV represents *dextro*-quercitol which is therefore, and perhaps significantly, a monodesoxy-*dextro*-inositol.

2. *Other Quercitols*

Power and Tutin[80] in 1904 reported the discovery of a polyhydric alcohol in the leaves of *Gymnema sylvestre* Br., one of the *Asclepiadaceae*. Elementary analysis of the compound and of its esters, as well as oxidation with permanganate to malonic acid, demonstrated the close relationship of the material to *dextro*-quercitol. However, this latter compound rotates +24° while the compound of Power and Tutin rotated −73.9° (for the hydrate) and so this levorotatory quercitol is not the enantiomorph of *dextro*-quercitol. As yet no information concerning its configuration has been published.

An interesting transition from the inositol to the quercitol series has recently been accomplished by Posternak.[64] While hydrogenation of *scyllo-meso*-inosose with platinum oxide as a catalyst in neutral solution gave the expected mixture of *meso*-inositol and scyllitol, it was found that, when the reduction was carried out in dilute sulfuric acid solution, two moles of hydrogen rather than one were consumed and the product was a desoxyinositol, presumably the symmetrical form XLIX. That this reaction does not proceed through an inositol is shown by the stability of *meso*-inositol under the conditions of the experiment.

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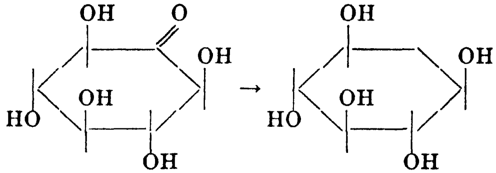
(80) F. B. Power and F. Tutin, *J. Chem. Soc.*, **85**, 624 (1904).



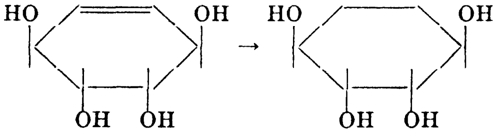
XXX
scyllo-meso-Inosose

XLIX

V. TETRAHYDROXYCYCLOHEXANES

1. *Dihydroconduritol*

Conduritol, a naturally occurring tetrahydroxycyclohexene (XXIV) has already been mentioned (page 58). Catalytic hydrogenation gives dihydroconduritol (L).[56,57]

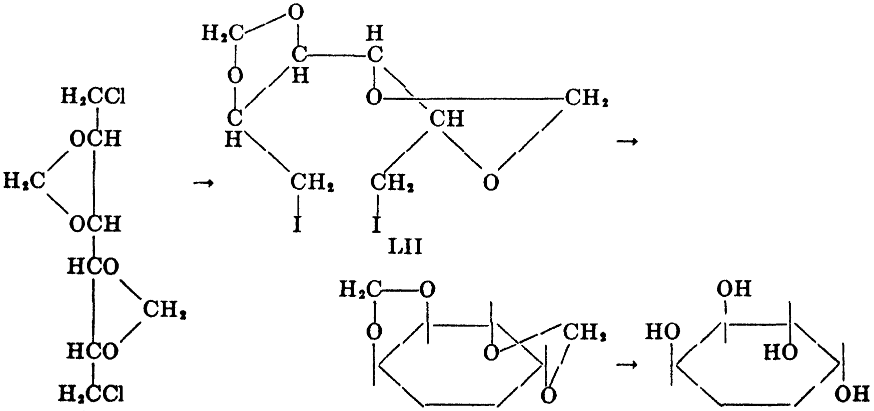


XXIV
Conduritol

L
Dihydroconduritol

2. *Tetrahydroxymannocyclitol*

As mentioned earlier, there has been no lack of attempts at synthesis in the cyclitol field. Work in this direction led Micheel[81] to an ingenious synthesis from D-mannitol of a tetrahydroxycyclohexane which he named "tetrahydroxymannocyclitol." The starting product was the previously



LI

LII

LIII

LIV
Tetrahydroxy-mannocyclitol

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(81) F. Micheel, *Ann.*, **496**, 77 (1932).

known 1,6-dichloro-1,6-didesoxy-D-mannitol, prepared by the direct treatment of D-mannitol with hydrochloric acid. Condensation of this product with formaldehyde gave a dimethylene derivative which, in view of past experience with cyclic acetals and of its stability, Micheel considered to be the 2,3:4,5-isomer (LI).

Sodium iodide in acetone at 115° converted this compound into the corresponding iodine derivative LII. It will be noted that, while the completely free rotation between the carbon-to-carbon bonds in 1,6-dichloro-1,6-didesoxy-D-mannitol renders the approach of one chlorine to the other somewhat unlikely, the situation is quite different in the substituted derivatives LI and LII. In these cases, the free rotation between carbon atoms two and three and between carbons four and five is restricted while rotation between carbon atoms three and four tends to bring the terminal halogen atoms in close proximity. It was with this reasoning in mind that Micheel treated his 1,6-diiodo-1,6-didesoxy-2,3:4,5-dimethylene-D-mannitol with "molecular" silver in xylene at 165–170°. Complete dehalogenation occurred and there was eventually isolated a dimethylene derivative of a tetrahydroxycyclohexane (LIII) in a yield of about 5%. Hydrolysis with hot concentrated hydrochloric acid removed the methylene residues and generated the optically active tetrahydroxymannocyclitol LIV. Supporting evidence for the assigned structure came: through zinc dust distillation, which furnished benzene; and through nitric acid oxidation, which yielded succinic acid.

3. *Betitol*

In 1901, von Lippmann[82] reported the discovery of an optically active tetrahydroxycyclohexane in beet sugar molasses. This polyol, which von Lippmann named betitol, has not been investigated further and, since it does not appear to be identical with any of the other known tetrahydroxycyclohexanes, both its structure and configuration are still wholly unknown.

4. *The Isomeric 1,2,3,5-Tetrahydroxycyclohexanes of Dangschat and Fischer*

Two optically active 1,2,3,5-tetrahydroxycyclohexanes have been obtained by Dangschat and Fischer[55] starting from a 4,5-isopropylidene-3,4,5-trihydroxy-1-cyclohexanone (LVI) derived from quinic acid (see page 72). Hydrogenation of this ketone in the presence of nickel, followed by acid hydrolysis gave one cyclitol while reduction with aluminum isopropoxide followed by hydrolysis, gave a second cyclitol. Since the position of the newly formed hydroxyl group with respect to the

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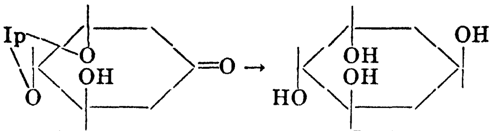
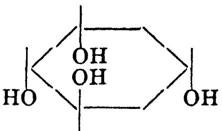
(82) E. O. von Lippmann, *Ber.*, **34**, 1159 (1901).



LVI
4,5-Isopropylidene-3,4,5-trihydroxycyclohexanone-1

LVII



LVIII

plane of the ring is not known, formulas LVII and LVIII remain open for these isomers.[83]

5. *Other Tetrahydroxycyclohexanes*

Various other tetrahydroxycyclohexanes have been reported as purely synthetic products. Bedos and Ruyer,[84] for example, found that the hydration of a compound which they considered to be 1,2,3,4-diepoxycyclohexane gave two isomeric tetrols. Zelinsky and Titowa,[85] in the hope of synthesizing betitol, hydroxylated 1,4-cyclohexadiene with aqueous permanganate at 0°. The high melting (241–242°) product which they obtained and named "cyclohexane-erythritol" was apparently a new substance as might be expected in view of its optical inactivity. Several years later, Zelinsky, Denisenko and Eventova[85] hydroxylated 1,3-cyclohexadiene in a similar manner to obtain yet another tetrol, presumably a stereoisomer of the compounds of Bedos and Ruyer.

At the present time nothing is known concerning the configuration of these purely synthetic tetrols although it would appear that no duplicates are involved.

VI. QUINIC AND SHIKIMIC ACIDS

In 1806 Vauquelin[86] isolated from cinchona bark an acidic crystalline substance which he named *acide kinique*. Later the same substance was reported in the coffee bean, in hay and in various other substances.[87] The ready conversion of quinic acid to aromatic substances early demonstrated its carbocyclic nature. Treatment with aqueous bromine gave protocatechuic acid[88]; with phosphorus pentachloride, *m*-chlorobenzoyl

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(83) It may be noted that since neither isomer is identical with betitol, von Lippmann's suggestion[82] that betitol may be configurationally related to quinic acid is disproved.

(84) P. Bedos and A. Ruyer, *Compt. rend.*, **196**, 625 (1933).

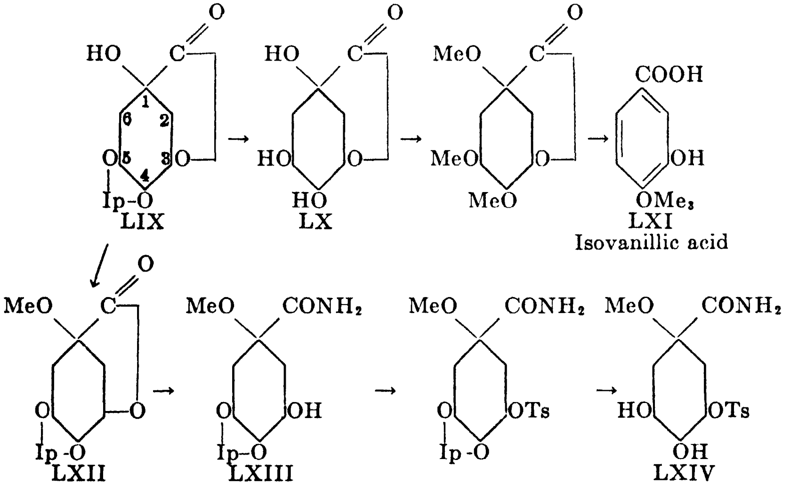
(85) N. D. Zelinsky and A. N. Titowa, *Ber.*, **64**, 1399 (1931); N. D. Zelinsky, J. I. Denisenko and M. S. Eventova, *Compt. rend. acad. sci. U. R. S. S.*, **1**, 313 (1935); *Chem. Zentr.*, **II**, 3765 (1935).

(86) L. N. Vauquelin, *Ann. chim.*, **59**, 162 (1806).

(87) Beilstein, 4th ed., **10**, 535.

(88) R. Fittig and T. Macalpine, *Ann.*, **168**, 99 (1873).

chloride was formed.[89] The presence of four hydroxyl groups was shown by acetylation to the triacetate of a lactone.[90] That at least one of these hydroxyl groups was attached to the same carbon as the carboxyl group, was made likely by the observation that warming with concentrated sulfuric acid gave carbon monoxide.[91] Active attack on the structure and configuration of quinic acid was begun by H. O. L. Fischer[92] in 1921 with the observation that acetone and hydrochloric acid converted the compound into the monoacetone derivative of its lactone (LIX). Fischer and Dangschat[93] used this derivative to prove both the structure and configuration of quinic acid. Hydrolysis of it with dilute acid gave the free lactone LX which was methylated and then treated with bromine water to give isovanillic acid (LXI). From these facts it may be reasoned that lactone formation involved a hydroxyl group on carbon three while condensation with acetone proceeded at carbon four and some other carbon. Methylation of the acetonated lactone gave a monomethyl derivative LXII which was converted with ammonia into the corresponding amide LXIII. The free hydroxyl group at carbon three was then converted to the *p*-toluenesulfonate and the acetone residue was removed by hydrolysis. The resulting compound LXIV was found to consume one mole of lead tetraacetate, demonstrating that a hydroxyl group is attached to carbon five. The remaining hydroxyl group must then be located on carbons one or two.



Ts = *p*-toluenesulfonyl

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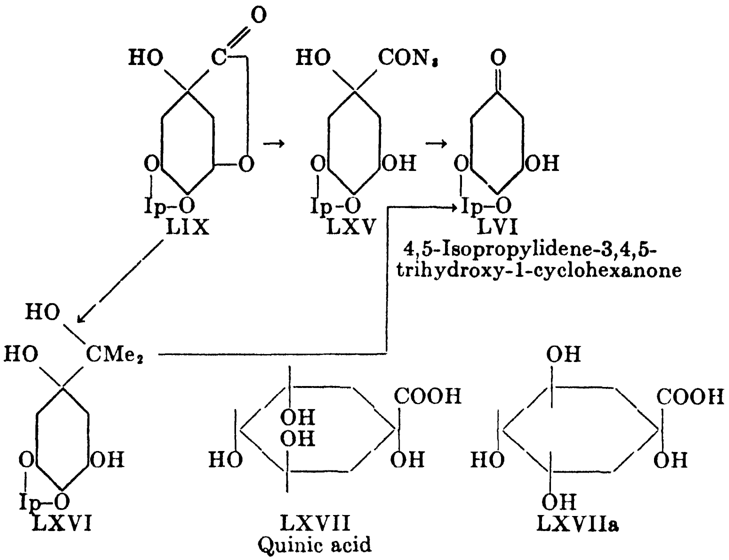
(89) C. Graebe, *Ann.*, **138**, 197 (1866).

(90) E. Erwig and W. Koenigs, *Ber.*, **22**, 1457 (1889).

(91) O. Hesse, *Ann.*, **110**, 194 (1859).

(92) H. O. L. Fischer, *Ber.*, **54**, 775 (1921).

(93) H. O. L. Fischer and Gerda Dangschat, *Ber.*, **65**, 1009 (1932).



Additional proof of the positions of the hydroxyl groups in quinic acid was obtained through two degradative processes. The acetone quinic acid lactone LIX was converted to the corresponding azide LXV which when heated yielded 4,5-isopropylidene-3,4,5-trihydroxy-1-cyclohexanone (LVI). The same compound was obtained by another procedure: treatment of acetone quinic acid lactone (LIX) with an excess of methyl magnesium iodide gave a glycol (LXVI) which, when cleaved by lead tetraacetate, also gave LVI. This 4,5-isopropylidene-3,4,5-trihydroxy-1-cyclohexanone was found to give a phenylhydrazone but no osazone. This fact, together with that of its formation, furnishes strong evidence for structure LVI.

The configuration of quinic acid was adduced as LXVII or its mirror image (LXVIIa) as follows:

1. The carboxyl group must lie on the same side of the plane of the ring as the hydroxyl group at carbon three, otherwise lactone formation between the two groups would be impossible.
2. The hydroxyl group attached to carbon one must therefore be *trans* to that on carbon three.
3. The hydroxyl groups on carbon atoms four and five must be *cis* to each other since they condense with acetone.
4. These same hydroxyls must be *trans* to the hydroxyl on carbon three since quinic acid is optically active and 3-methylquinic acid does not form a lactone.

Shikimic acid, a very close relative of quinic acid, was discovered by Eijkman[94] in the Chinese star anise (*Illicium religiosum* Sieb.) and various other similar plants.[95] Preliminary investigation[96] showed the compound to be a trihydroxytetrahydrobenzoic acid. The actual structure was elucidated by Fischer and Dangschat[97] who hydrogenated methyl shikimate and then oxidized the resulting product LXVIII with periodic acid followed by bromine water. Hydrolysis then gave tricarballylic acid—proving dihydroshikimic acid to have the structure LXVIII. The following year the same authors reported[98] the more delicate degradation of methyl shikimate to *trans*-aconitic acid. Since dihydroshikimic acid is optically active it follows that the hydroxyl groups on carbon atoms three and five in shikimic acid must be *trans* to one another. From this it can be seen that shikimic acid has configuration LXIX or LXX (or their respective enantiomorphs LXIXa and LXXa).

In 1937, Fischer and Dangschat[99] published an elegant and final proof of the configuration of shikimic acid. Acetonation and esterification of shikimic acid gave a compound which, in the form of its acetate (LXXI), was hydroxylated by potassium permanganate in neutral solution to LXXII. Dilute alkali removed the methyl and acetyl groups from this compound to give LXXIII which was then cleaved with periodic acid. The product, presumably LXXIV, together with its cyclic tautomer, was not isolated as such but was treated in two separate ways. First, reaction with one mole of bromine in dilute acetic acid solution in the presence of silver acetate gave LXXV which, when hydrolyzed with acid and reduced catalytically, gave the known 2-desoxy-D-gluconic acid (LXXVI). Since such a product could only come from LXIX, this formula must represent shikimic acid. A second synthetic path confirmed this conclusion. Treatment of LXXIV with sodium hypobromite gave the lactone LXXVII which, with an excess of methyl magnesium iodide, was converted to LXXVIII. This alcohol proved inert to the oxidizing action of periodic acid. If shikimic acid had structure LXX or LXXa, a compound such as LXXIX would have been obtained and would have been readily oxidizable by periodic acid.

The very close relationship of quinic acid to shikimic acid was demonstrated in 1938 by Dangschat and Fischer[100] through the conversion of quinic into shikimic acid. 3-Acetyl-4,5-methylenequinic amide (LXXX)

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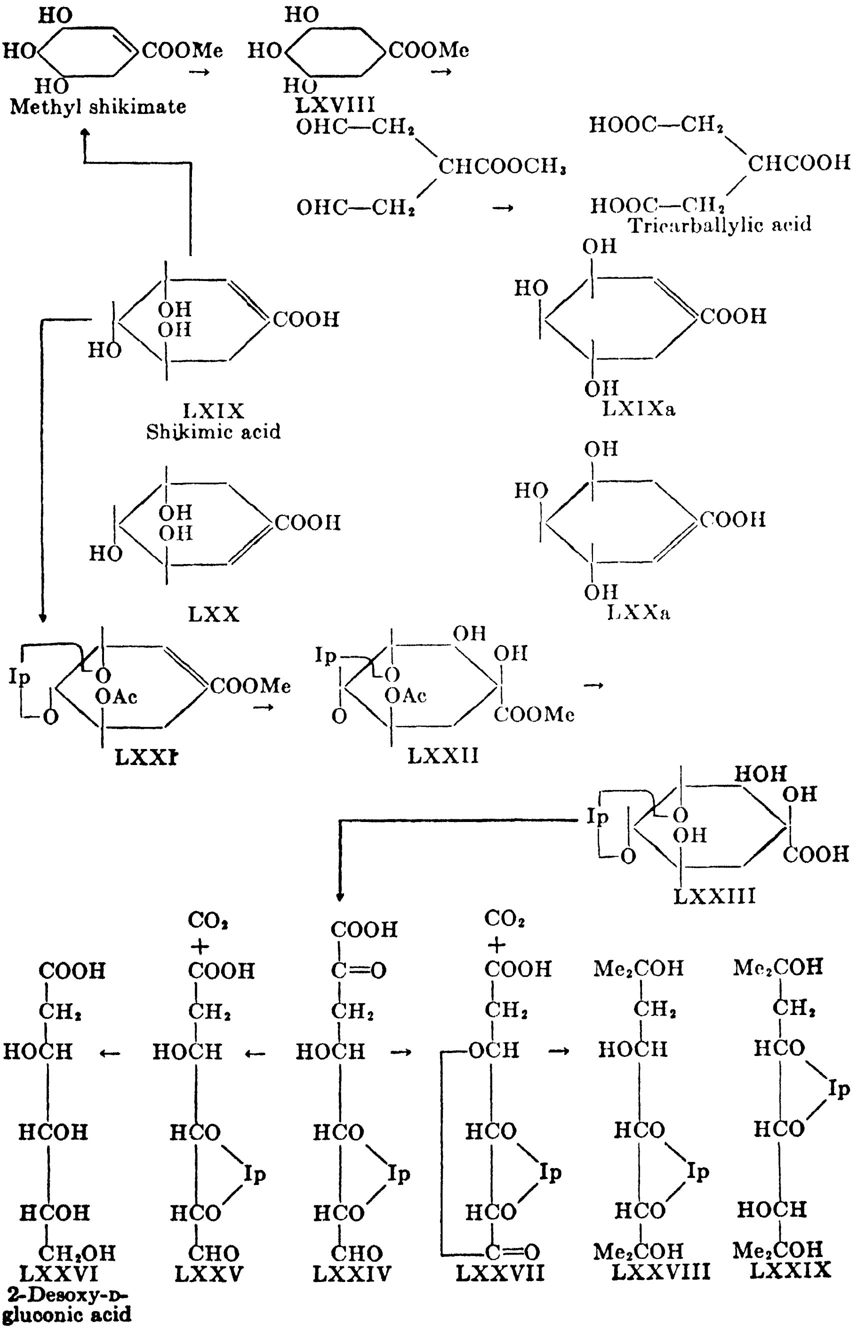
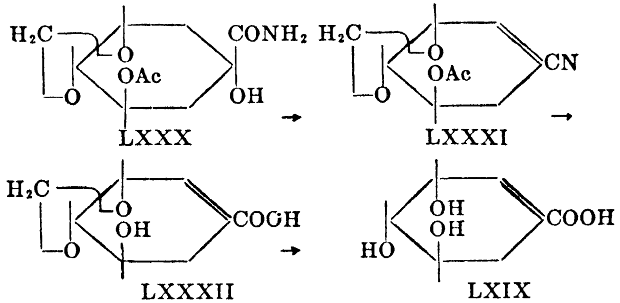
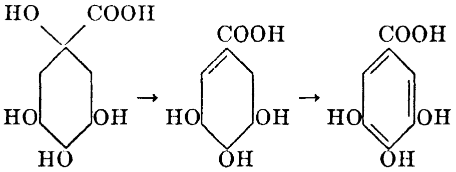
(94) J. F. Eijkman, *Rec. trav. chim.*, **4**, 32 (1885).
(95) J. F. Eijkman, *Rec. trav. chim.*, **5**, 299 (1886).
(96) J. F. Eijkman, *Ber.*, **24**, 1278 (1891).
(97) H. O. L. Fischer and Gerda Dangschat, *Helv. Chim. Acta*, **17**, 1200 (1934).
(98) H. O. L. Fischer and Gerda Dangschat, *Helv. Chim. Acta*, **18**, 1204 (1935).
(99) H. O. L. Fischer and Gerda Dangschat, *Helv. Chim. Acta*, **20**, 705 (1937).
(100) Gerda Dangschat and H. O. L. Fischer, *Naturwissenschaften*, **26**, 562 (1938).


Methyl shikimate
LXVIII
OHC—CH₂
CHCOOCH₃
OHC—CH₂
HOOC—CH₂
CHCOOH
HOOC—CH₂
Tricarballylic acid
LXIX
Shikimic acid
LXIXa
LXX
LXXa
LXXI
LXXII
LXXIII
COOH
C=O
CH₂
HOCH
HCO
Ip
CHO
CO₂
+
LXXVI
2-Desoxy-D-gluconic acid
LXXV
LXXIV
LXXVII
LXXVIII
LXXIX
Me₂COH

was treated with an excess of *p*-toluenesulfonyl chloride and pyridine to give 3-acetyl-4,5-methyleneshikimic nitrile (LXXXI) which, on hydrolysis, gave 4,5-methyleneshikimic acid (LXXXII) and, finally, shikimic acid (LXIX). This synthesis establishes the configuration of quinic acid as LXVII (rather than LXVIIa) and shows that quinic acid is configurationally related to D-glucose.


LXXX LXXXI LXXXII LXIX

The striking configurational relationship between D-glucose, quinic acid, shikimic acid and gallic acid strongly suggests a biogenetic relationship among these compounds.[98,100]


Quinic acid Shikimic acid Gallic acid

That quinic acid is the precursor of shikimic acid is made more probable by the recent discovery of both acids in the carpels of *Illicium verum* Hook.[101] Other possible roles of quinic acid in nature have been discussed by Hall.[102]

VII. MYTILITOL AND DERIVATIVES

Jansen[103] in 1913 and Ackermann[104] in 1921 independently reported the discovery of a polyalcohol in the edible mussel, *Mytilus edulis*. This substance was named mytilitol and was considered by its first discoverer to be an isomer of quercitol and by its second discoverer to be a C-methyl inositol. Daniel and Doran[105] several years later reviewed the evidence, which they considered to favor Ackermann's interpretation.

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(101) A. Boldt, *Pharm. Zentralhalle*, **78**, 157 (1937).
(102) J. A. Hall, *Chem. Revs.*, **20**, 305 (1937).
(103) B. C. P. Jansen, *Z. physiol. Chem.*, **85**, 231 (1913).
(104) D. Ackermann, *Ber.*, **54**, 1938 (1921).
(105) R. J. Daniel and W. Doran, *Biochem. J.*, **20**, 676 (1926).

In 1944 the structure and configuration of the compound were determined by Posternak.[106] Confirmation of the C-methyl inositol structure for mytilitol was obtained by chromic acid oxidation to acetic acid. Recourse was then had to synthesis starting from *scyllo-meso*-inosose (XXX). Either of the pentaacetates of this compound,[107] treated with a large excess of methylmagnesium iodide, was found to give mytilitol (LXXXIII) and a small quantity of a new, isomeric cyclitol that was named isomytilitol (LXXXIV). Much better yields of this latter cyclitol were obtained by treating *scyllo-meso*-inosose pentaacetate with diazomethane. There resulted a cyclic oxide (LXXXV) which, on catalytic hydrogenation and hydrolysis, gave isomytilitol in excellent yield. Finally, it was found that a similar synthesis could be effected from free *scyllo-meso*-inosose through the oxide LXXXVI. In order to ascertain which of these isomeric cyclitols corresponded to configurations LXXXIII and LXXXIV, recourse was had to periodic acid oxidation. Just as *meso*-inositol was found to be oxidized more rapidly than its completely *trans* isomer, scyllitol, so isomytilitol was found to consume periodic acid more rapidly than mytilitol. Formula LXXXIII therefore represents mytilitol and LXXXIV isomytilitol; the former may be considered as a C-methylscyllitol, the latter as 5-C-methyl-*meso*-inositol.[108] The cyclic oxide LXXXV proved a fruitful starting point for a series of reactions. With acetic acid in the presence of a catalyst such as sodium acetate, it readily changed to the hexaacetate LXXXVII, which possessed a free hydroxyl group. That this hydroxyl group is a tertiary one was indicated by its resistance to acetylation. The structure of LXXXVII was further demonstrated by reaction of the original cyclic oxide LXXXV with *p*-toluenesulfonic acid to give the *p*-toluenesulfonate LXXXVIII. Like the corresponding acetate, this ester contained a hydroxyl group resistant to acetylation. That the tosyloxy group was attached to the primary carbon atom was evident by its ready replacement by iodine. Treatment of the *p*-toluenesulfonate with potassium acetate gave LXXXVII which could also be synthesized from the cyclic oxide LXXXV by addition of hydrogen bromide (to form LXXXIX) and subsequent treatment with potassium acetate. Hydrolysis of the hexaacetate LXXXVII yielded a new cyclitol (XC). The question arises whether XC is configurationally related to mytilitol or isomytilitol. It will be

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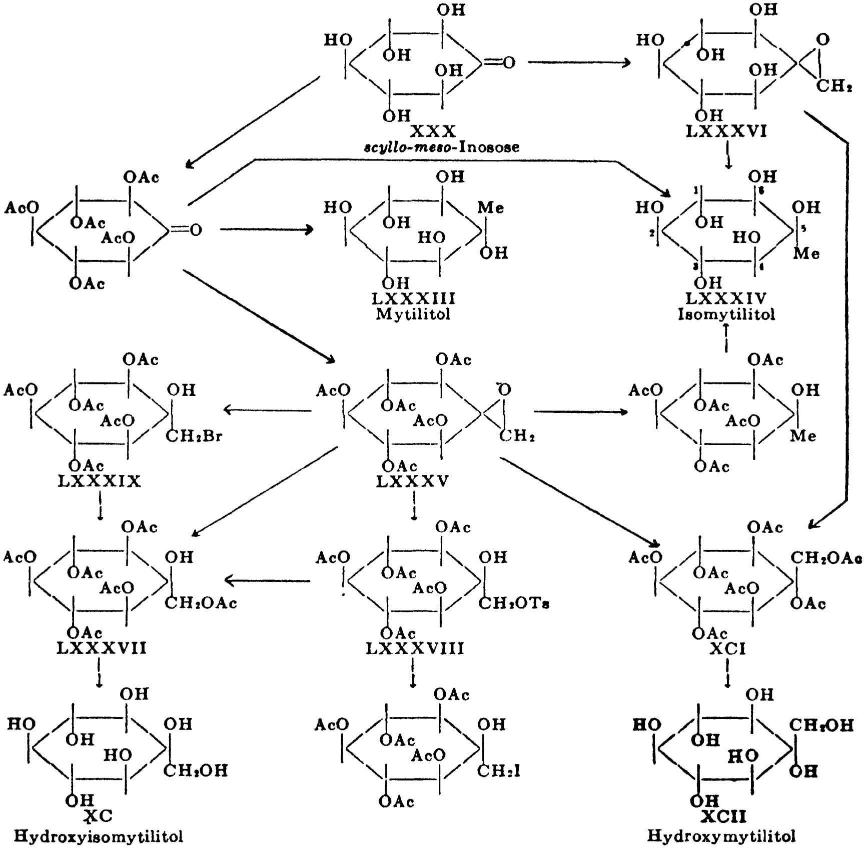
(106) T. Posternak, *Helv. Chim. Acta*, **27**, 457 (1944).

(107) Two mutually interconvertible pentaacetates and two pentabenzoates of *scyllo-meso*-inosose were obtained by Posternak, ref. 64. Whether these represent cases of dimorphism or some other variety of isomerism has not as yet been ascertained.

(108) Since all the carbon atoms in scyllitol are equivalent, no number is necessary to designate the position of a single substituent. This is not the case with *meso*-inositol and the numbers have been so assigned as to emphasize the relationship of the compound to D-glucose.

recalled that catalytic reduction of the cyclic oxide LXXXV furnished isomytilitol by a mechanism which presumably involved the opening of the oxygen-methylene bond. Since no asymmetric center is disturbed in the process it is safe to assume that the cyclic oxide is an isomytilitol derivative and that acids add to it, as did hydrogen, without Walden inversion. The new cyclitol XC is therefore hydroxyisomytilitol.



If the cyclic oxide LXXXV, on the other hand, be treated with acetic anhydride in the presence of ferric chloride or zinc chloride there is formed a heptaacetate (XCI). The same compound is obtained by acetylation of the cyclic oxide LXXXVI in the presence of ferric chloride or zinc chloride. Hydrolysis of this heptaacetate yielded XCII, an isomer of hydroxyisomytilitol. It is apparent that a Walden inversion has occurred here in the opening of both cyclic oxides; XCII is therefore appropriately named hydroxymytilitol.

TRITYL ETHERS OF CARBOHYDRATES

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I. INTRODUCTION

The triphenylmethyl ethers of the carbohydrates constitute a group of derivatives of particular interest because of the special properties inherent in the trityl group.[1] Before entering into a discussion of these carbohydrate derivatives, a few words regarding the preparation, properties and special behavior of trityl ethers would appear to be in order.

Trityl ethers may be prepared from tritanol by reaction with an alcohol in the presence of an acid catalyst[2] or from trityl chloride by reaction with an alcohol[3] or an alkoxide.[4] Reaction 1 is reversible and is

$$\mathrm{TrOH + ROH \rightleftarrows TrOR + H_2O} \qquad (1)$$

$$\mathrm{TrCl + ROH \rightleftarrows TrOR + HCl} \qquad (2)$$

$$\mathrm{TrCl + ROK \rightarrow TrOR + KCl} \qquad (3)$$

---

(1) In accordance with general practice, trityl will be used for the equivalent term triphenylmethyl; tritanol for triphenylmethanol; tritane for triphenylmethane; tosyl for *p*-toluenesulfonyl; and mesyl for methanesulfonyl. The abbreviations Tr, Ts and Ms represent the trityl, *p*-tosyl and mesyl groups, respectively.

(2) E. J. Salmi and E. Renkonen, *Ber.*, **72**, 1107 (1939).

(3) C. Friedel and J. M. Crafts, *Ann. chim. phys.*, [6] **1**, 503 (1884).

(4) A. Baeyer, *Ber.*, **42**, 2625 (1909).

similar to esterification, with tritanol playing the part of an acid. The equilibrium may be shifted to the right in reaction 1 by removal of the water (azeotropic distillation with benzene[2]) and in reaction 2 by removal of the hydrogen chloride (aeration with an inert gas[5]). In the latter reaction the trityl chloride behaves in a manner analogous to an acid chloride.

The tritylation of carbohydrates is carried out in absolute pyridine,[6,7] with which trityl chloride, like a true acid chloride, forms an addition compound. The latter, on treatment with a carbohydrate containing an available hydroxyl group[8] yields the corresponding trityl ether and pyridine hydrochloride. Many carbohydrates are soluble in pyridine, an additional advantage attending the use of this solvent, which is generally present in considerable excess.[9] The velocity of a tritylation reaction depends upon the nature of the hydroxyl-containing component[10] and the temperature, varying in the case of primary hydroxyl groups, from several days at room temperature to a few hours at 100°. Since the reaction can be carried out in the absence of an appreciable concentration of water or acid, it is applicable to such acid-sensitive substances as the glycosides.

Most trityl ethers crystallize well — a welcome property in the carbohydrate group. They do, however, tend to form mixed crystals with other trityl compounds present in solution, and to form addition complexes with such solvents as alcohol and pyridine.[11] Their purification therefore requires close attention. The distillation of a trityl ether has not been reported; the high temperature required involves the danger of substituent group migration, as well as more deep-seated rearrangements.[12,13,14] The purification of trityl ethers is usually accomplished by recrystallization, preferably from different solvents, although chromatography has been used with success.[15]

Trityl ethers are nearly insoluble in water or petroleum ether, slightly soluble in ether and the lower alcohols, and more easily soluble in benzene,

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(5) S. Sabetay, *Compt. rend.*, **203**, 1164 (1936).

(6) B. Helferich, P. E. Speidel and W. Toeldte, *Ber.*, **56**, 766 (1923).

(7) B. Helferich, L. Moog and A. Jünger, *Ber.*, **58**, 872 (1925).

(8) The tritylation velocity (availability) of primary and secondary hydroxyl groups is discussed on page 86.

(9) F. Ebel, *Ber.*, **60**, 2079 (1927).

(10) R. C. Hockett and M. L. Downing, *J. Am. Chem. Soc.*, **64**, 2463 (1942).

(11) M. L. Wolfrom, W. J. Burke and S. W. Waisbrot, *J. Am. Chem. Soc.*, **61**, 1827 (1939).

(12) C. D. Hurd and E. M. Filachione, *J. Am. Chem. Soc.*, **59**, 1949, 1952 (1937).

(13) J. F. Norris and R. C. Young, *J. Am. Chem. Soc.*, **46**, 2582 (1924).

(14) J. F. Norris and R. C. Young, *J. Am. Chem. Soc.*, **52**, 753 (1930).

(15) K. Zeile and W. Kruckenberg, *Ber.*, **75**, 1127 (1942).

chloroform or pyridine. The predominant effect of the hydrophobic trityl group is indicated in the low water solubility of trityl ethers containing free hydroxyl groups. Petroleum ether is useful for the separation of trityl ethers from tritane,[16,17] while ether and the lower alcohols are most often used as recrystallization solvents. The rotation of optically active trityl ethers is usually determined in benzene, chloroform or pyridine.

In their behavior toward acids and bases, trityl ethers resemble the acetals, glycosides and orthoesters, being fairly stable in alkali, but easily split by acidic reagents.[4] Thus, in cases where carbonyl compounds are used to protect pairs of hydroxyl groups by the formation of acetals, tritylation may often be used to protect suitably situated individual hydroxyl groups.

Trityl ethers are easily cleaved to tritanol and another alcohol (detritylation) by strong acids at room temperature. In water, the rate of cleavage of trityl ethers is low because of their low solubility. It may be increased by the addition of such solvents as ethanol and acetone. Trityl ethers are cleaved in good yield by boiling in 80% acetic acid for one-half hour.[18] A very rapid cleavage is obtained by dissolving the trityl ether in concentrated sulfuric acid and pouring this solution into water. A quantitative assay for the trityl group[19] is based on this detritylation procedure.

Alcoholysis of trityl ethers occurs very readily. The reaction requires only traces of acid. Acidic substances must therefore be rigidly excluded from recrystallization solvents if losses due to detritylation are to be avoided. Wolfrom, Burke, and Waisbrot[11] found that ditrityldulcitol prepared in the usual manner with pyridine and trityl chloride crystallized with two moles of pyridine hydrochloride. When the addition compound was recrystallized from ethanol, the acidity of the pyridine hydrochloride was sufficient to saponify the ditrityldulcitol completely. To avoid detritylation, the pyridine hydrochloride was removed before recrystallization.

Detritylation can also be carried out in non-hydroxylic solvents.[16,20,21]

---

(16) P. E. Verkade, W. D. Cohen and A. K. Vroege, *Rec. trav. chim.*, **59,** 1123 (1940); P. E. Verkade, F. D. Tollenaar and T. A. P. Posthumus, *ibid.*, **61,** 373 (1942).

(17) P. E. Verkade, J. van der Lee. J. C. de Quant and E. de Roy van Zuydewijn, *Proc. Acad. Sci. Amsterdam,* **40,** 580 (1937).

(18) R. Kuhn, H. Rudy and F. Weygand, *Ber.*, **69,** 1546 (1936).

(19) F. Valentin, *Collection Czechoslov. Chem. Communs.*, **3,** 499 (1931); *Chem. Zentr.*, **I,** 2160 (1932).

(20) B. Helferich and H. Koester, *Ber.*, **57,** 587 (1924).

(21) P. E. Verkade, J. van der Lee and W. Meerburg, *Rec. trav. chim.*, **54,** 716 (1935).

Chloroform or ether solutions of hydrogen chloride smoothly cleave trityl ethers to trityl chloride and an alcohol. This reaction is the reverse of one of the methods for preparing trityl ethers (reaction 2, page 79). If the detritylated product is difficultly soluble in chloroform or ether, as are most carbohydrates, it precipitates during detritylation and is easily separated from the trityl chloride.

Hydrogen bromide in glacial acetic acid has also been used to split trityl ethers.[22] Since trityl bromide is very slightly soluble in acetic acid, it precipitates during detritylation, leaving the alcohol in solution. In the presence of excess hydrogen bromide, the detritylated product is sometimes converted to the corresponding bromide.[11,23]

$$\mathrm{TrOR} + 2\mathrm{HBr} \rightarrow \mathrm{TrBr} + \mathrm{RBr} + \mathrm{H_2O} \qquad (4)$$

In detritylation with phosphorus pentabromide,[24] an alkyl bromide, trityl bromide and phosphorus oxybromide are formed. This reaction may be used to locate the position of the trityl group.[7] Phosphorus pentachloride reacts in a similar manner.

Catalytic reduction in the presence of platinum[24a] or palladium[16] is a very mild method for splitting trityl ethers. The products are an alcohol and tritane. The latter may be separated from the alcohol by taking advantage of its solubility in petroleum ether. If the trityl ether contains sulfur, poisoning of the platinum or palladium catalyst may occur.[25]

The qualitative analysis of trityl compounds takes advantage of the orange-yellow color of the halochromic salt formed when a trityl compound is treated with a solution of concentrated sulfuric acid in glacial acetic acid.

Quantitative analysis is carried out in one of several ways depending on the solubility of the detritylated compound. If the latter is insoluble in an organic solvent which dissolves the trityl ether, a weighed sample of the ether (tritylcellulose,[20] for example) is dissolved in a solvent such as chloroform, and a saturated solution of hydrogen chloride in chloroform is added. The solvent must be anhydrous and free of alcohol. The detritylated compound separates rapidly and is removed by filtration, washed with chloroform, dried and weighed. The combined chloroform solutions are then evaporated to dryness, and the weight of the trityl chloride residue is determined.

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(22) B. Helferich and W. Klein, *Ann.*, **450**, 219 (1926).

(23) M. L. Wolfrom, J. L. Quinn and C. C. Christman, *J. Am. Chem. Soc.*, **56**, 2789 (1934).

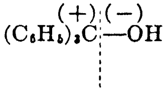
(24) B. Helferich, W. Klein and W. Schäfer, *Ann.*, **447**, 19 (1926).

(24*a*) F. Micheel, *Ber.*, **65**, 262 (1932).

(25) H. W. Arnold and W. L. Evans, *J. Am. Chem. Soc.*, **58**, 1950 (1936).

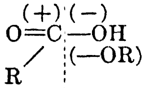
If the detritylated compound is soluble in water, a weighed sample of the trityl ether is dissolved in a small amount of concentrated sulfuric acid. After a short time at room temperature, the solution is poured into water and the precipitated tritanol is filtered, washed with water, dried and weighed. This method has the advantage of speed and simplicity of operation, and gives accurate results.[19]

The great ease of formation as well as the facile and peculiar splitting of trityl ethers can be explained by the unusual nature of the trityl group. Tritanol is a tertiary alcohol. In distinction to primary and secondary alcohols, it has a tendency to react by splitting out the entire hydroxyl group together with its binding electrons. The hydroxyl group of a tertiary alcohol is less firmly bound than that of a primary or secondary alcohol. This fission into an hydroxyl ion is especially enhanced in tritanol (as is the splitting out of OR in trityl ethers) by the strong tendency of the trityl group to appear as a trityl cation. This tendency so greatly weakens the bond between the methane carbon of the trityl group and the oxygen in tritanol (or its ether) that the ease of its scission is comparable to the removal of oxygen from a carbon which is simultaneously bound to several oxygen atoms, as in acids, esters, aldehyde or ketone hydrates, hemiacetals or acetals.



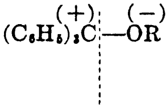
$\overset{(+)}{(C_6H_5)_3C}\vdots\overset{(-)}{—OH}$

Tritanol



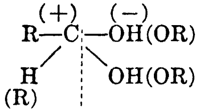
$\overset{(+)}{O{=}C}\vdots\overset{(-)}{—OH}$ (—OR), R

Acid (ester)



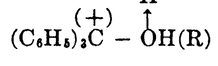
$\overset{(+)}{(C_6H_5)_3C}\vdots\overset{(-)}{—OR}$

Trityl ether



Aldehyde (ketone) hydrate,
hemiacetal or acetal

This tendency toward formation of a trityl cation is brought to completion by the addition of a proton (H^+) to the oxygen atom of the tritanol or trityl ether.



$$\overset{(+)}{(C_6H_5)_3C} - \overset{\overset{H^+}{\uparrow}}{O}H(R)$$

The trityl cation may then react with Cl^- or Br^- by salt formation to form the halide,

$$(C_6H_5)_3\overset{+}{C} + Cl^- \rightarrow (C_6H_5)_3CCl$$

or with an alkoxide ion to form the ether.

$$(C_6H_5)_3\overset{+}{C} + OR^- \rightarrow (C_6H_5)_3COR$$

Hydrolysis in the presence of water and acids may be explained likewise.

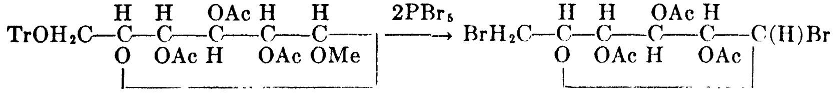
$$(C_6H_5)_3\overset{+}{C} + OH^- \rightarrow (C_6H_5)_3COH$$

The indifference of trityl ethers toward alkalies is explainable by the fact that alkali causes little or no formation of the trityl cation. This, in turn, is because OH^- (in contradiction to H^+) does not add to an ether oxygen or to an hydroxyl oxygen. With esters, addition of the hydroxyl ion occurs, due to the presence of the carbonyl group.

II. Carbohydrate Trityl Ethers[26]

The first trityl ethers of free monosaccharides were the 6-trityl ethers of D-glucose and D-galactose.[7] They were prepared from the sugar and trityl chloride in pyridine solution. The yield of only 30–50% indicates that the reaction proceeds in several different directions when only one mole of trityl chloride is used. Probably the lactol hydroxyl group on carbon atom 1, as well as the C6 primary hydroxyl group, becomes tritylated.[15] If the hydroxyl in the 1-position is protected, as in methyl α- or β-D-glucosides, the reaction gives a much better yield.[27]

With few exceptions,[28] the formation of all known trityl ethers of the aldohexoses and their derivatives involves the hydroxyl in the 6-position. For example, treatment of methyl 6-trityl-2,3,4-triacetyl-α-D-glucopyranoside (I) with phosphorus pentabromide replaces the tritoxyl group by bromine and leads to 2,3,4-triacetyl-6-bromo-6-desoxy-D-glucopyranosyl bromide[7,24] (II).



$$TrOH_2C\text{—}\overset{H}{\underset{O}{C}}\text{—}\overset{H}{\underset{OAc}{C}}\text{—}\overset{OAc}{\underset{H}{C}}\text{—}\overset{H}{\underset{OAc}{C}}\text{—}\overset{H}{\underset{OMe}{C}}\xrightarrow{2PBr_5} BrH_2C\text{—}\overset{H}{\underset{O}{C}}\text{—}\overset{H}{\underset{OAc}{C}}\text{—}\overset{OAc}{\underset{H}{C}}\text{—}\overset{H}{\underset{OAc}{C}}\text{—}C(H)Br$$

I

II

6-Trityl-β-D-mannose,[29] 6,6′-ditrityltrehalose,[30] and 1,6-ditrityl-D-fructose[31] were prepared later. All these trityl ethers of free sugars contained solvent of crystallization. Ditritylfructose crystallized with two molecules of pyridine,[31] and 1,6-ditrityldulcitol with two molecules of pyridine hydrochloride.[11]

A systematic examination of the tritylation of pentoses by Zeile and Kruckenberg[15] resulted in the isolation of ditrityl ethers of D-xylose and

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(26) B. Helferich, *Z. angew. Chem.*, **41,** 871 (1928).

(27) B. Helferich and Johanna Becker, *Ann.*, **440,** 1 (1924).

(28) R. C. Hockett, H. G. Fletcher, Jr., and J. B. Ames, *J. Am. Chem. Soc.*, **63,** 2516 (1941).

(29) B. Helferich and J. F. Leete, *Ber.*, **62,** 1549 (1929).

(30) H. Bredereck, *Ber.*, **63,** 959 (1930).

(31) B. Helferich, *J. prakt. Chem.*, [2] **147,** 60 (1936).

L-arabinose, and a monotrityl ether of D-xylose. The latter had the trityl group attached glycosidically at carbon atom 1, since it did not reduce Fehling solution and showed no mutarotation.

A large number of sugar alcohols have also been tritylated (see page 110). Most of these trityl ethers are well crystallized substances. Very likely the tritylation has occurred at the primary hydroxyl groups. Pentaerythritol gave a crystalline tetratrityl ether melting above 350° and having a molecular weight of 1104.5.[19]

Many trityl ethers of sugars and sugar alcohols have been isolated as the acetates or benzoates. Generally these derivatives are more easily purified than the unacylated compounds.

The solubility of carbohydrate trityl ethers is quite different from that of carbohydrates. Sugars, sugar alcohols and other simple polyhydroxy compounds are generally soluble only in water and pyridine, and to a far lesser degree in the lower alcohols or acids. Tritylation changes the solubility markedly. Trityl ethers are soluble in many organic solvents such as chloroform, benzene and acetone, even when there are several free hydroxyl groups in the molecule. On the other hand, their solubility in water is negligible. The trityl ethers of polysaccharides also dissolve in chloroform, forming colloidal solutions.

The solubility of the trityl ethers is of advantage in experimental work. For example,[32] during the conversion of methyl 2,3,4-triacetyl-β-D-glucopyranoside to the 2,3,6-triacetate (see page 95) there is formed an equilibrium mixture of these two substances from which the compound with the free 4-hydroxyl group can be separated only in poor yield by simple crystallization. Separation is more complete when the mixture is tritylated in the ordinary way with trityl chloride in pyridine. Tritylation occurs almost exclusively at the 6-hydroxyl of the 2,3,4-triacetate, the 6-trityl ether thus formed being insoluble in water. The untritylated 2,3,6-triacetate can then be extracted and is obtained in good yield. It is of interest as an intermediate in the synthesis of cellobiose.[32]

It may be of advantage in certain reactions to protect individual hydroxyl groups by tritylation. For example, Bredereck found that the mutarotation of D-ribose proceeded in a different manner than that of its 5-trityl ether. With the latter, the mutarotation corresponded to an α-β transformation of the two furanose forms. In free ribose more than two forms participate in the mutarotation, probably the two pyranose forms in addition to the two furanose forms. The possible formation of a pyranose ring is excluded by tritylation[33] in the 5-position.

Another example of the successful blocking of hydroxyl groups by

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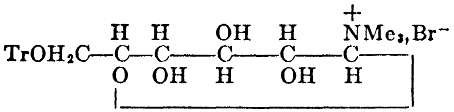
(32) B. Helferich and H. Bredereck, *Ber.*, **64**, 2411 (1931).

(33) H. Bredereck, M. Köthnig and Eva Berger, *Ber.*, **73**, 956 (1940).

tritylation was investigated by Micheel.[34] Ordinarily, (β-D-glucopyranosyl)-trimethylammonium bromide yields levoglucosan on treatment with alkali. However, Micheel found that ring closure was inhibited when the 6-hydroxyl group was tritylated. Thus (6-trityl-β-D-glucopyranosyl)-trimethylammonium bromide (III) did not give levoglucosan but rather methyl 6-trityl-β-D-glucopyranoside, when treated with alcoholic sodium hydroxide.



$$\mathrm{TrOH_2C{-}\underset{O}{\overset{H}{C}}{-}\underset{OH}{\overset{H}{C}}{-}\underset{H}{\overset{OH}{C}}{-}\underset{OH}{\overset{H}{C}}{-}\underset{H}{\overset{\overset{+}{N}Me_3,Br^-}{C}}{-}}$$

III

The use of such tritylation methods may prove to be of importance with other sugars and sugar derivatives.

III. Rate of Tritylation of Primary and Secondary Hydroxyl Groups

The tritylation velocity of methyl α-D-glucopyranoside has been measured quantitatively.[35] Using two moles of trityl chloride in an excess of pyridine at 30°, the tritylation is bimolecular. After five to six hours, tritylation is 98% complete. The participation of hydroxyl groups other than the primary one has not been observed.

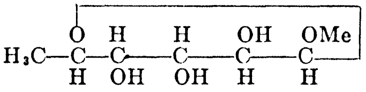
The simple alcohols whose trityl ethers have been prepared by treatment with trityl chloride and pyridine include, in addition to primary alcohols, 2-propanol,[6] cyclohexanol,[6] *l*-menthol,[36] *d*-borneol,[36] and cholesterol.[36] Trityl ethers involving the secondary hydroxyl group in glycerol are also known.[37,38] There can be no doubt, therefore, that secondary as well as primary hydroxyl groups react with trityl chloride in pyridine.

On the other hand, experience has shown that in polyhydroxy compounds containing both primary and secondary hydroxyl groups, the primary hydroxyls react preferentially. As a rule, particularly in the absence of a significant excess of trityl chloride, only the trityl ethers of primary hydroxyl groups are isolated. This fact has led to the assumption that the reaction with trityl chloride in pyridine may be used for establishing the presence of primary hydroxyl groups in polyhydroxy compounds. The formation of a trityl ether was thought to be conclusive

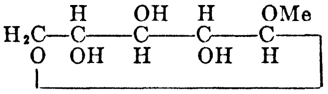
(34) F. Micheel and Hertha Micheel, *Ber.*, **65**, 258 (1932).
(35) B. Helferich and N. M. Bigelow, *J. prakt. Chem.*, [2] **131**, 259 (1931).
(36) K. Josephson, *Ann.*, **493**, 174 (1931).
(37) A. Fairborne and G. W. Cowdrey, *J. Chem. Soc.*, 129 (1929).
(38) P. E. Verkade and J. van der Lee, *Rec. trav. chim.*, **55**, 267 (1936).

proof for the presence of one or more primary hydroxyl groups. However, this conclusion should be drawn only with considerable caution, even though it has usually led to correct results and to the discovery of primary hydroxyls in unexpected instances.[39,40]

In 1931, Hockett and Hudson[41] reported that pentosides without primary hydroxyls reacted with trityl chloride in pyridine. In later papers, these authors described the preparation of a monotrityl ether of methyl α-L-fucopyranoside[42] (IV), and of derivatives of the mono and ditrityl ethers of methyl β-D-xylopyranoside (V).

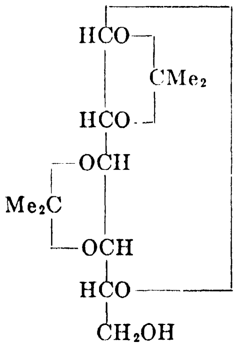


IV

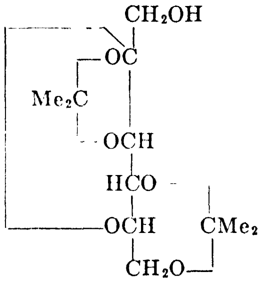


V

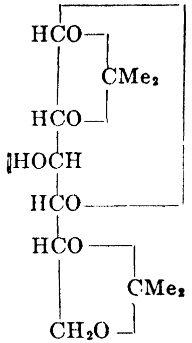
In all these instances tritylation occurred at the secondary hydroxyl groups since there were no primary hydroxyl groups present. Because of the stability of the glycosides, rearrangements during the reaction were ruled out. Subsequently, the tritylation of 1,2:3,4-diisopropylidene-D-galactose (VI), 2,3:4,6-diisopropylidene-L-sorbose (VII), and 1,2:5,6-diisopropylidene-D-glucofuranose (VIII) was investigated quantitatively by Hockett and coworkers.[28]



VI



VII



VIII

All three compounds yielded crystalline trityl ethers. However, a considerable difference in tritylation velocity was found. With four moles

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(39) F. Micheel and K. Hasse, *Ber.*, **68**, 1582 (1935).

(40) J. W. Oldham and J. K. Rutherford, *J. Am. Chem. Soc.*, **54**, 366 (1932).

(41) R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, **53**, 4456 (1931).

(42) R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 945 (1934).

of trityl chloride at room temperature, the reaction constants calculated for a pseudo-monomolecular reaction were: 0.014 and 0.0052, respectively, for compounds VI and VII, with primary hydroxyls; and 0.00012 for substance VIII, with a secondary hydroxyl. The ratio is 116.6:43.3:1. The differences are made even clearer by considering the time required for half-tritylation. The primary hydroxyls of compounds VI and VII are half-tritylated after 21.6 and 57.9 hours, respectively, while the secondary hydroxyl of substance VIII is half-tritylated only after 2,500 hours. On simultaneous tritylation of compounds VII and VIII, using four moles of trityl chloride, 50% of compound VII and only 1.6% of compound VIII would be tritylated after 57.9 hours. When eight moles of trityl chloride were used, only slightly different figures were obtained.

These results demonstrate the different reaction velocity of the two primary hydroxyl groups and the markedly lower reaction velocity of the secondary hydroxyl group. In another paper,[10] it was shown that the same relative difference in reaction velocity is observed on treating compounds VII and VIII with *p*-toluenesulfonyl chloride, and hence that the nature of the hydroxyl group and not that of the chloride is responsible for these differences. Without precise knowledge of the difference in reaction rates, this behavior had been used previously for the selective tosylation,[43] mesylation[44,45] and qualitative estimation[5] of primary hydroxyl groups. However, the preparation of pure substances is easier when tritylation is employed.

Studies of the tritylation velocity of primary and secondary hydroxyl groups have led to the following conclusions, on the premise that the reaction is carried out at room temperature for one to two days (or on the water-bath for one to two hours) and only a small excess of trityl chloride is used.

(1) If an hydroxyl group does not react with trityl chloride in pyridine, it is not a primary hydroxyl group. No instance of an unreactive primary hydroxyl group has yet been reported.

(2) If a polyhydroxy compound contains both primary and secondary hydroxyl groups, the primary groups react preferentially.

(3) If a compound contains only free secondary hydroxyl groups these may be tritylated.

(4) If a polyhydroxy compound in which the positions of the hydroxyl groups are unknown, readily yields one or more trityl ethers, this reaction is an indication, though not a definite proof, of the presence of primary

(43) J. Compton, *J. Am. Chem. Soc.*, **60**, 395 (1938).

(44) B. Helferich and A. Gnüchtel, *Ber.*, **71**, 712 (1938).

(45) B. Helferich, H. Dressler and R. Griebel, *J. prakt. Chem.*, [2] **153**, 285 (1939).

hydroxyl groups. For more precise conclusions, a quantitative determination of tritylation velocity is recommended.

(5) Trityl ethers of primary hydroxyl groups may be characterized as such. One method includes reaction with phosphorus pentabromide or hydrogen bromide to form the corresponding bromide. Another involves detritylation followed by conversion of the free hydroxyl group to a tosyl or mesyl group. If the tosyl or mesyl compound thus formed reacts with sodium iodide in acetone to form an iododesoxy compound, a primary hydroxyl group is indicated, according to Oldham and Rutherford.[40] Although this reaction is quite conclusive with polyhydroxy compounds, tosyl and mesyl esters of simple secondary alcohols also give iodides when treated with sodium iodide.[46]

The rules given above must be kept in mind when one critically examines published data concerning the detection of primary hydroxyl groups by tritylation. In the absence of better proof, tritylation studies may be used for detecting primary hydroxyl groups, but the results must be interpreted with caution.

The C6 position of the trityl group in the first trityl ethers of D-glucose and the D-glucosides was established by transformation into 2,3,4-triacetyl-6-bromo-6-desoxy-D-glucopyranosyl bromide or into other derivatives of 6-bromo-6-desoxy-D-glucose (see page 84). Brigl and Grüner[47] found that a certain dibenzoyl-D-mannitol could not be tritylated under ordinary conditions. The absence of free primary hydroxyl groups was therefore assumed, and the compound was designated as 1,6-dibenzoyl-D-mannitol.

Tritylation at the primary hydroxyl group in 1-(5-trityl-D-ribofuranosyl)-1,2,3,4-tetrahydropyrimidine-2,4-dione (monotrityluridine) was established by Levene and Tipson.[48] The two free hydroxyl groups were first esterified with *p*-toluenesulfonic acid. Since the trityl-ditosyl compound thus formed did not react with sodium iodide[40] it did not contain a primary tosyl group. Therefore, tritylation had occurred at the primary hydroxyl group.

In experiments designed to clarify the structure of a component of certain bacterial polysaccharides, Goebel and coworkers[49] established the position of the free primary hydroxyl group in methyl 2,4-dimethyl-β-D-glucopyranoside by tritylation, followed by tosylation and reaction with sodium iodide.

(46) B. Helferich and E. Günther, *Ber.*, **72**, 338, 392 (1939).

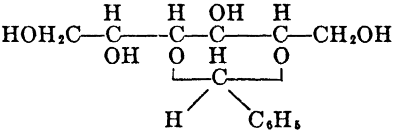
(47) P. Brigl and H. Grüner, *Ber.*, **65**, 641 (1932).

(48) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **105**, 419 (1934).

(49) M. H. Adams, R. E. Reeves and W. F. Goebel, *J. Biol. Chem.*, **140**, 653 (1941).

By tritylation studies, Bredereck[50,51] established the presence of a free primary hydroxyl group and a furanose ring in the D-ribose portion of certain nucleosides. This proof is quite conclusive because of comparative experiments with D-glucopyranosyltheophylline, whose primary hydroxyl group easily forms a trityl ether,[50] and with methyl D-ribopyranoside which, in the absence of a primary hydroxyl group, is not tritylated under ordinary conditions.[50]

The following example shows that tritylation may reveal primary hydroxyl groups even in unexpected instances. The formula originally suggested for monobenzylidenesorbitol contained only one primary hydroxyl group. Micheel and Hasse[39] isolated a ditrityl ether, even when only one mole of trityl chloride was used. They concluded therefore, that two primary hydroxyl groups were present and by independent means were able to establish that the monobenzylidenesorbitol in question had structure IX.



$$\begin{array}{c}HOH_2C-\underset{OH}{\overset{H}{C}}-\underset{O}{\overset{H}{C}}-\underset{H}{\overset{OH}{C}}-\underset{O}{\overset{H}{C}}-CH_2OH\\ \text{(2,4-O-)}\;CH(C_6H_5)\end{array}$$

IX

However, in a number of other examples where the formation of trityl ethers was used as evidence for the presence of primary hydroxyl groups, the data must be interpreted with caution.

IV. TRITYL INTERMEDIATES IN THE SYNTHESIS OF CARBOHYDRATES

1. *Partially Acylated Carbohydrates*

Tritylation protects individual hydroxyl groups against the action of alkali. The remaining hydroxyl groups may then be esterified and the tritylated hydroxyl group, which in most instances is a primary one, may be regenerated by careful detritylation. A particularly important example of the preparation of partially acylated sugars was the synthesis of 1,2,3,4-tetraacetyl-D-glucopyranose.[22,52] D-Glucose, when treated with trityl chloride in pyridine, yielded 6-trityl-D-glucopyranose (X), which was then acetylated in the reaction mixture by treatment with acetic anhydride. The resulting 6-trityl-1,2,3,4-tetraacetyl-β-D-glucopyranose (XI) was detritylated with hydrogen bromide in glacial acetic acid to 1,2,3,4-tetraacetyl-β-D-glucopyranose (XII) having a free hydroxyl group in the 6-position. The latter compound has been useful in various

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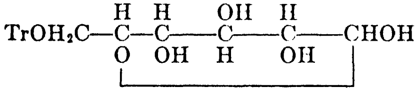
(50) H. Bredereck, *Ber.*, **65**, 1830 (1932).

(51) H. Bredereck, *Ber.*, **66**, 198 (1933).

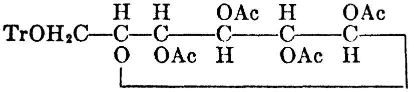
(52) D. D. Reynolds and W. L. Evans, *J. Am. Chem. Soc.*, **60**, 2559 (1938).

syntheses. The 2,3,4-triacetyl-D-glucopyranosyl chloride, made from levoglucosan by Zemplén,[53] may be used similarly.



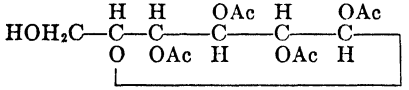
$TrOH_2C-\overset{H}{\underset{O}{C}}-\overset{H}{\underset{OH}{C}}-\overset{OH}{\underset{H}{C}}-\overset{H}{\underset{OH}{C}}-CHOH$

X



$TrOH_2C-\overset{H}{\underset{O}{C}}-\overset{H}{\underset{OAc}{C}}-\overset{OAc}{\underset{H}{C}}-\overset{H}{\underset{OAc}{C}}-\overset{OAc}{\underset{H}{C}}-$

XI



$HOH_2C-\overset{H}{\underset{O}{C}}-\overset{H}{\underset{OAc}{C}}-\overset{OAc}{\underset{H}{C}}-\overset{H}{\underset{OAc}{C}}-\overset{OAc}{\underset{H}{C}}-$

XII

Other partially acylated sugars and sugar derivatives containing a free primary hydroxyl group were prepared in a similar manner using as starting materials: methyl α-D-glucopyranoside,[54] methyl β-D-glucopyranoside,[55,54] D-mannose,[29] methyl α-D-mannopyranoside,[56] riboflavin,[18] D-mannitol[57] and L-fucitol.[11] Partially acylated derivatives with a free primary hydroxyl group were also prepared from sugar mercaptals and from aldehydo sugars by Micheel and Spruck[53] and by Wolfrom and coworkers.[23,59] Trehalose[30] and methyl β-cellobioside,[60] have also been partially acylated by the above method.

Migration of the acyl group has frequently been observed in these instances. A careful investigation was carried out with 1,2,3,4-tetraacetyl-β-D-glucopyranose (XII). The 4-acetyl group in this compound migrates to the 6-position,[24,61,62] the compound (XIV) containing a free hydroxyl group at C4 appearing as a byproduct in the preparation of XII. The orthoacetic ester (XIII) is probably an intermediate in this reaction; the ring closure is possible without strain despite the *trans* position of the substituents at the C4 and C5. A triacetyl derivative with a free

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(53) G. Zemplén, *Ber.*, **68**, 1318 (1935).

(54) B. Helferich, H. Bredereck and A. Schneidmüller, *Ann.*, **458**, 111 (1927).

(55) J. W. Oldham, *J. Chem. Soc.*, **127**, 2844 (1925).

(56) A. J. Watters, R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1528 (1939).

(57) F. Micheel, *Ann.*, **496**, 77 (1932).

(58) F. Micheel and W. Spruck, *Ber.*, **67**, 1665 (1934).

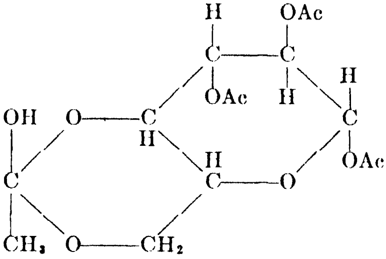
(59) M. L. Wolfrom, J. L. Quinn and C. C. Christman, *J. Am. Chem. Soc.*, **58**, 39 (1936).

(60) B. Helferich, E. Bohm and S. Winkler, *Ber.*, **63**, 989 (1930).

(61) B. Helferich and W. Klein, *Ann.*, **455**, 173 (1927).

(62) B. Helferich and A. C. Müller, *Ber.*, **63**, 2142 (1930).

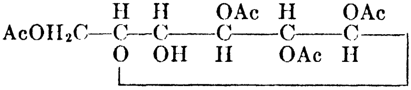
4-hydroxyl group was prepared in a similar manner from methyl β-D-glucopyranoside.[32,54,62] However, these substances are of limited use for further synthetic work since the secondary 4-hydroxyl group reacts very slowly.[32] Other partially acetylated compounds behave similarly, the



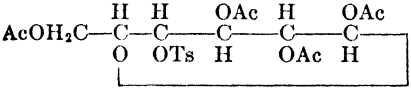
XIII

migration of an acetyl group to a primary hydroxyl group leading to acetyl derivatives with one free secondary hydroxyl group.

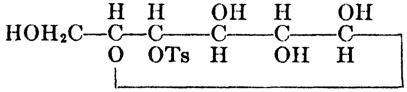
All such compounds having one free hydroxyl group, or several in the case of oligosaccharides, can be esterified at this hydroxyl group. By removing the acyl radicals first introduced, without involving the acyl radical introduced subsequently, it is possible to synthesize partially acylated sugars containing only one acyl group. Thus, 4-tosyl-β-D-glucopyranose[62] (XVI) was obtained from 1,2,3,6-tetraacetyl-β-D-glucopyranose (XIV) by tosylation (XV) and subsequent deacetylation.



XIV



XV



XVI

A similar reaction sequence led to the synthesis of riboflavin 5-phosphate[18] and, earlier, to the 6-phosphate of methyl α-D-glucopyranoside.[63]

The following types of partially acylated carbohydrates may be prepared with the aid of tritylation.

(1) Carbohydrates with one free primary hydroxyl group.

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(63) B. Helferich and H. du Mont, *Z. physiol. Chem.*, **181,** 300 (1929).

(2) Carbohydrates with one free hydroxyl group; in this case, the one whose acyl group has migrated to a free primary hydroxyl group.

(3) Carbohydrates with one acyl group located at a primary hydroxyl group.

(4) Carbohydrates with one acyl group situated at the hydroxyl group liberated through acyl migration.

(5) Carbohydrates in which the above possibilities are correspondingly increased because of the presence of several primary hydroxyl groups.

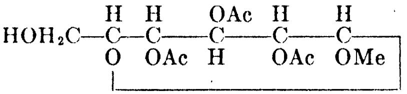
(6) Carbohydrates prepared from intermediate trityl ethers of secondary hydroxyl groups. These occur less frequently.

2. *Oligosaccharides*

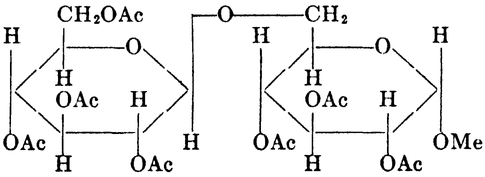
An old problem in the chemistry of carbohydrates has been the synthesis of oligosaccharides from monosaccharides, if possible in a way which proves the structure and configuration of the oligosaccharides. This problem has been solved in some instances with starting materials made available through tritylation.

The first compounds synthesized in this manner were methyl α-gentiobioside and gentiobiose itself.[22,27,64]

Methyl 2,3,4-triacetyl-α-D-glucopyranoside (XVII) was condensed with 2,3,4,6-tetraacetyl-D-glucopyranosyl bromide to form methyl hepta-acetyl-α-D-gentiobioside (XVIII) and the latter deacetylated to free methyl α-gentiobioside (XIX). Free gentiobiose (XXII) was obtained by using 2,3,4-tribenzoyl-D-glucopyranosyl fluoride (XX) and, later, 1,2,3,4-tetraacetyl-β-D-glucopyranose (XXI) for the condensation with 2,3,4,6-tetraacetyl-D-glucopyranosyl bromide.

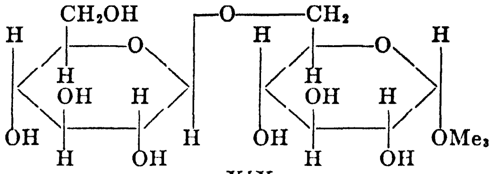


XVII

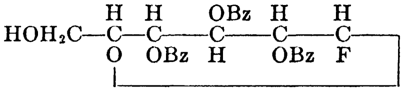


XVIII

(64) B. Helferich, K. Bäuerlein and F. Weigand, *Ann.*, **447**, 27 (1926).

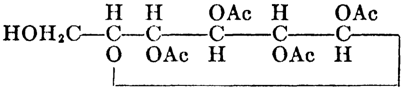


XIX



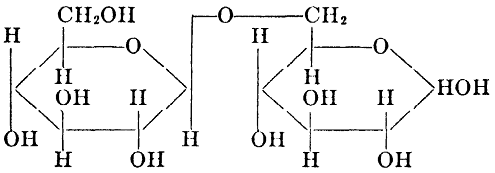
$$\mathrm{HOH_2C{-}\underset{O}{\overset{H}{C}}{-}\underset{OBz}{\overset{H}{C}}{-}\underset{H}{\overset{OBz}{C}}{-}\underset{OBz}{\overset{H}{C}}{-}\underset{F}{\overset{H}{C}}}$$

XX

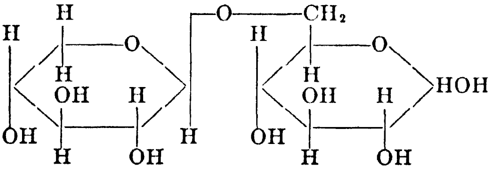


$$\mathrm{HOH_2C{-}\underset{O}{\overset{H}{C}}{-}\underset{OAc}{\overset{H}{C}}{-}\underset{H}{\overset{OAc}{C}}{-}\underset{OAc}{\overset{H}{C}}{-}\underset{H}{\overset{OAc}{C}}}$$

XXI



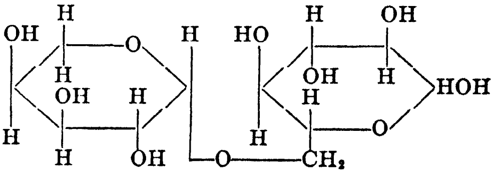
XXII



XXIII

Thus, for the first time, a natural disaccharide was synthesized in a manner proving both structure and configuration. This synthesis was developed into a preparative method by Reynolds and Evans.[52]

The free 6-hydroxyl group of 1,2,3,4-tetraacetyl-D-glucopyranose (XXI) has been used in other syntheses of reducing oligosaccharides. Condensation with triacetyl-D-xylopyranosyl bromide led to primeverose, a sugar which occurs rather frequently in plants. This reaction proved the structure and configuration of primeverose to be 6-(β-D-xylopyranosyl)-D-glucopyranose[65] (XXIII).

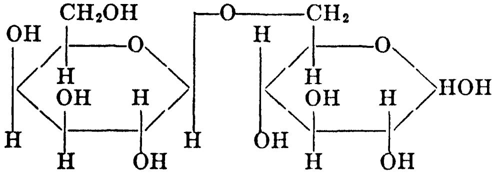


XXIV

[65] B. Helferich and H. Rauch, *Ann.*, **465**, 168 (1927).

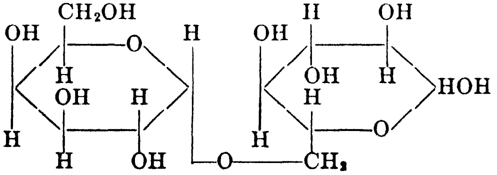
Natural vicianose was synthesized in like manner by condensing 1,2,3,4-tetracetyl-D-glucopyranose with triacetyl-L-arabopyranosyl bromide; it structure is therefore 6-(β-L-arabopyranosyl)-D-glucopyranose[66] (XXIV).

In a similar manner, tetraacetyl-D-galactopyranosyl bromide yielded 6-(β-D-galactopyranosyl)-D-glucopyranose (XXV) which was shown to be probably identical with allolactose from breast milk.[67,68]



XXV

Melibiose was similarly obtained through condensation with tetraacetyl-D-galactopyranosyl bromide in quinoline.[66] This synthesis established the identity of the sugar with 6-(α-D-galactopyranosyl)-D-glucopyranose (XXVI), both structurally and configurationally.



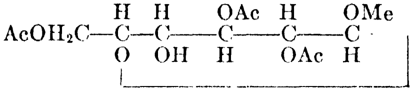
XXVI

In addition to these five naturally occurring disaccharides there were obtained by similar syntheses the disaccharide 1-(β-D-glucopyranosyl)-D-fructose,[66] three trisaccharides,[69] and two tetrasaccharides.[66,70]

The constitution and configuration of cellobiose was confirmed by synthesizing the sugar, though in very small yield. This was the first synthesis of cellobiose by way of a trityl compound. Methyl 2,3,6-triacetyl-β-D-glucopyranoside (XXVII) was condensed with tetraacetyl-D-glucopyranosyl bromide and the condensation product identified as methyl heptaacetyl-β-cellobioside (XXVIII). Since the latter compound yields acetobromocellobiose (heptaacetylcellobiosyl bromide) and cello-

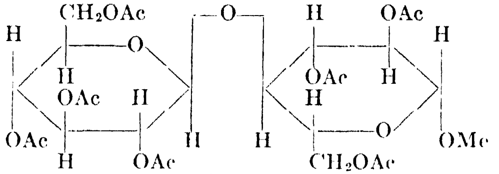
(66) B. Helferich and H. Bredereck, *Ann.*, **465**, 166 (1928).
(67) B. Helferich and H. Rauch, *Ber.*, **59**, 2655 (1926).
(68) B. Helferich and G. Sparmberg, *Ber.*, **66**, 806 (1933).
(69) B. Helferich and W. Schäfer, *Ann.*, **450**, 229 (1926).
(70) B. Helferich and R. Gootz, *Ber.*, **64**, 109 (1931).

biose,[32,71] the disaccharide was identified as 4-(β-D-glucopyranosyl)-D-glucopyranose.



$$\begin{array}{ccccccccccc} & & \text{H} & & \text{H} & & \text{OAc} & & \text{H} & & \text{OMe}\\ \text{AcOH}_2\text{C} & - & \text{C} & - & \text{C} & - & \text{C} & - & \text{C} & - & \text{C}\\ & & \text{O} & & \text{OH} & & \text{H} & & \text{OAc} & & \text{H}\end{array}$$

XXVII



XXVIII

It is likely that other partially acylated carbohydrates which are obtainable by the tritylation method and contain only one free hydroxyl group (particularly if this is a primary one), may also be used for the synthesis of oligosaccharides.

Trityl ethers of the higher polysaccharides are also known, the trityl ethers of cellulose,[20] starch[20] and glycogen[72] having been reported.

3. *Partially Alkylated Carbohydrates*

In carbohydrate trityl ethers and the partially acylated carbohydrate derivatives obtained from them, the free hydroxyl groups may be etherified with other alcohols. This method has been used repeatedly, particularly for the preparation of partially methylated compounds. With the alkali resistant trityl ethers, any alkaline alkylation method may be used, for example, dimethyl sulfate and alkali, or methyl iodide and silver oxide. In choosing an alkylation method for partially acylated compounds, one must be selected which does not cause acyl migration or saponification.

In this manner[56] 6-methyl-D-glucopyranose[56] (XXIX) and some of its derivatives[27,73] were synthesized from D-glucose derivatives containing a free hydroxyl group only in the 6-position. The 6-methyl ethers of D-mannose and of methyl α-D-mannoside were obtained similarly. Haworth and coworkers[74] synthesized 2,3,4-trimethyl-D-glucose from 6-trityl-D-glucose and used the ether for the elucidation of structural problems.

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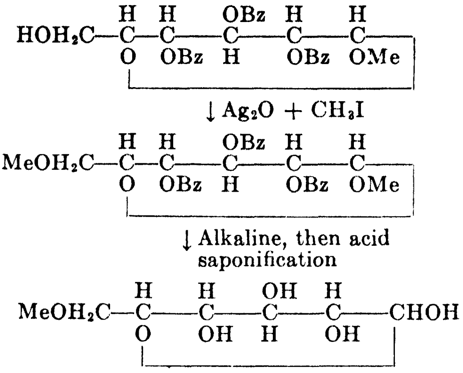
(71) G. Zemplén, *Ber.*, **53**, 996 (1920).

(72) L. Schmidt and E. Kotter, *Monatsh.*, **59**, 335 (1937).

(73) B. Helferich and E. Günther, *Ber.*, **64**, 1276 (1931).

(74) W. Charlton, W. N. Haworth and W. J. Hickinbottom, *J. Chem. Soc.*, 1529 (1927).



XXIX

The 6-trityl ether of methyl 3-tosyl-D-glucopyranoside was employed by Goebel and coworkers[49] for the unequivocal synthesis of methyl 2,4-dimethyl-β-D-glucopyranoside. This synthesis was an important link in elucidating the structure of the capsular polysaccharide of Type III pneumococcus.

For the structural clarification of euxanthic acid, Robertson and Waters[75] prepared methyl 2,3,4-trimethyl-α-D-glucopyranoside from the 6-trityl ether.

Many partially alkylated carbohydrates may be obtained in an analogous manner by using trityl ethers and the partially acylated carbohydrates obtainable from them.

4. *Carbohydrate Acetals*

In carbohydrate chemistry, condensation with carbonyl compounds such as acetone, acetaldehyde, formaldehyde and benzaldehyde is of great importance for the protection of pairs of hydroxyl groups. Tritylation may also be used in these instances for the protection of individual primary hydroxyl groups before carrying out the condensation with carbonyl compounds. After the condensation, the individual hydroxyl groups may be liberated by suitable detritylation methods. As yet, these possibilities have been explored very little. Wolfrom and coworkers[11] described the condensation of 1,6-ditrityldulcitol with benzaldehyde to 1,6-ditrityl-2,3,4,5-dibenzylidenedulcitol. Acetals other than those obtained by direct condensation are thus available.

5. *Halogen Derivatives of Carbohydrates*

By use of an appropriate reagent, the trityl group of a trityl ether may be replaced by a halogen atom. By means of this reaction, there may be

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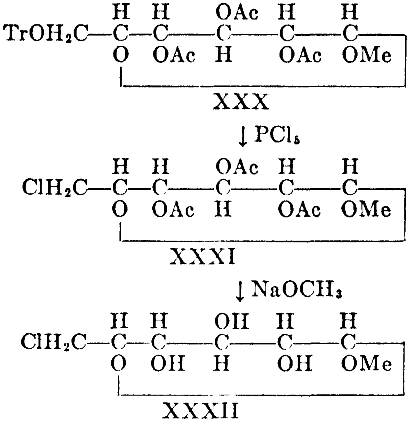
(75) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 1709 (1931).

synthesized a number of halogen-containing carbohydrates which could otherwise be obtained only with difficulty, if at all. A case in point is the preparation of methyl 6-chloro-6-desoxy-α-D-glucopyranoside (XXXII) which was used in the investigation of yeast α-glucosidase.[76] Methyl 6-trityl-2,3,4-triacetyl-α-D-glucopyranoside (XXX), on reaction with phosphorus pentachloride, splits off the trityl radical as trityl chloride and yields the corresponding 6-chloro-6-desoxy derivative (XXXI), which gives methyl 6-chloro-6-desoxy-α-D-glucopyranoside[77] (XXXII) on careful deacetylation.



$$\mathrm{TrOH_2C}-\overset{\mathrm{H}}{\underset{\mathrm{O}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OAc}}{\mathrm{C}}}-\overset{\mathrm{OAc}}{\underset{\mathrm{H}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OAc}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OMe}}{\mathrm{C}}}$$

XXX

$$\downarrow \mathrm{PCl_5}$$

$$\mathrm{ClH_2C}-\overset{\mathrm{H}}{\underset{\mathrm{O}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OAc}}{\mathrm{C}}}-\overset{\mathrm{OAc}}{\underset{\mathrm{H}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OAc}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OMe}}{\mathrm{C}}}$$

XXXI

$$\downarrow \mathrm{NaOCH_3}$$

$$\mathrm{ClH_2C}-\overset{\mathrm{H}}{\underset{\mathrm{O}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}-\overset{\mathrm{OH}}{\underset{\mathrm{H}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{OMe}}{\mathrm{C}}}$$

XXXII

The transformation of a β-D-glucoside into an α-D-glucoside was observed for the first time[78] by an analogous treatment of methyl 6-trityl-2,3,4-triacetyl-β-D-glucopyranoside with phosphorus pentabromide. The possibility of such anomerizations must be taken into account when using the phosphorus pentahalides. It is therefore important that other means be available for the preparation of halodesoxy carbohydrates from trityl ethers. One such method involves detritylation, followed by tosylation of the free hydroxyl group. If the resulting tosyl ester is primary, it may often be readily converted to the corresponding iodide by heating with sodium iodide in acetone.

This type of reaction has also been successful in more complicated instances.[30,60] For example, methyl β-cellobioside was converted to the 6,6′-ditrityl ether (XXXIII), the latter was acetylated (XXXIV), then detritylated (XXXV). The two primary hydroxyl groups were then esterified (XXXVI) with *p*-toluenesulfonyl chloride in pyridine and the ditosyl ester was converted to the diiodide (XXXVII) by treatment with

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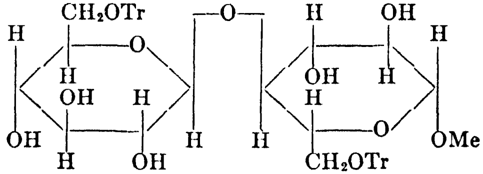
(76) B. Helferich, W. Klein and W. Schäfer, *Ber.*, **59**, 79 (1926).

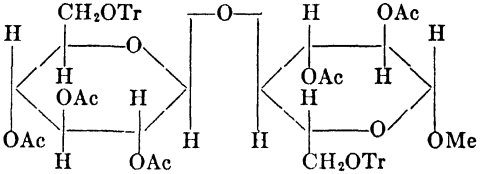
(77) B. Helferich and H. Bredereck, *Ber.*, **60**, 1995 (1927).

(78) B. Helferich and A. Schneidmüller, *Ber.*, **60**, 2002 (1927).

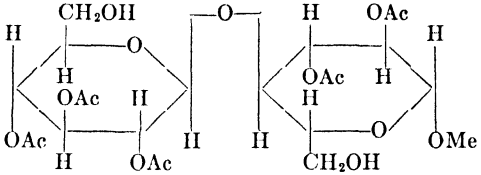
sodium iodide in acetone. Bredereck[30] prepared 2,3,4,2′,3′,4′-hexaacetyl-6,6′-diiodo-6,6′-didesoxytrehalose (XXXVIII) in a similar manner.



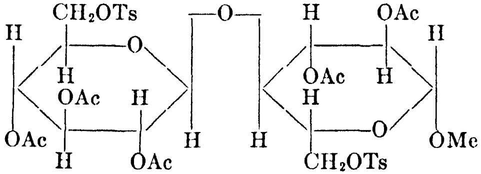
XXXIII



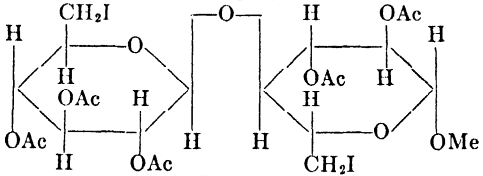
XXXIV



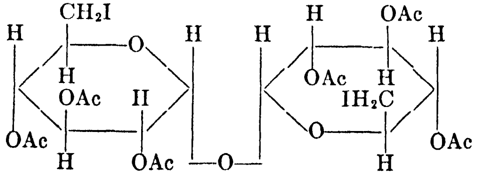
XXXV



XXXVI



XXXVII



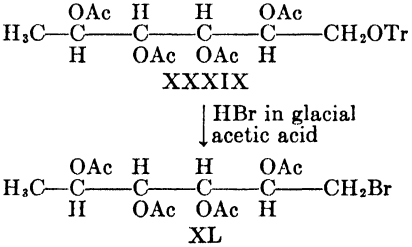
XXXVIII

Instead of *p*-toluenesulfonyl chloride, methanesulfonyl chloride may be used advantageously for esterification. Mesyl esters ordinarily react even more rapidly with sodium iodide than do tosyl esters.[44]

Terminally fluorinated carbohydrates may be obtained by treating the tosyl esters, or the more reactive mesyl esters, with potassium fluoride in methanol. Because of the alkaline reaction of potassium fluoride, the remaining hydroxyl groups must be protected, as for example, by acetal formation.

Müller[79] converted D-mannitol into the 1,6-diiodide as follows. 1,6-Ditrityl-D-mannitol was benzoylated and then detritylated; the free hydroxyl groups of the resulting 2,3,4,5-tetrabenzoyl-D-mannitol were esterified with *p*-toluenesulfonyl chloride and the 1,6-ditosyl-2,3,4,5-tetrabenzoyl-D-mannitol thus formed was treated with sodium iodide in acetone to form the diiododidesoxy tetrabenzoate.

Wolfrom and coworkers[11,23,59,79a] discovered another simple method which may be used occasionally for the transformation of trityl ethers into bromides. It was found that in certain instances detritylation with hydrogen bromide in glacial acetic acid did not free the primary hydroxyl groups but led to the corresponding bromides. Thus, 1-trityl-2,3,4,5-tetraacetyl-L-fucitol (XXXIX) gave the bromide (XL). 1,6-Dibromo-1,6-didesoxydulcitol 2,3,4,5-tetraacetate (XLII) was similarly obtained from 1,6-ditrityl-2,3,4,5-tetraacetyldulcitol (XLI) by treatment with hydrogen bromide in glacial acetic acid.



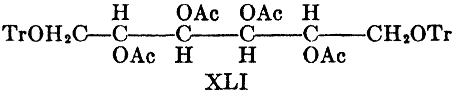
$$H_3C\text{—}\underset{H}{\overset{OAc}{C}}\text{——}\underset{OAc}{\overset{H}{C}}\text{——}\underset{OAc}{\overset{H}{C}}\text{——}\underset{H}{\overset{OAc}{C}}\text{——}CH_2OTr$$

XXXIX

$\downarrow$ HBr in glacial acetic acid

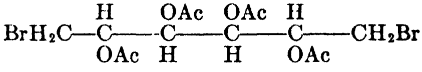
$$H_3C\text{—}\underset{H}{\overset{OAc}{C}}\text{——}\underset{OAc}{\overset{H}{C}}\text{——}\underset{OAc}{\overset{H}{C}}\text{——}\underset{H}{\overset{OAc}{C}}\text{——}CH_2Br$$

XL



$$TrOH_2C\text{—}\underset{OAc}{\overset{H}{C}}\text{——}\underset{H}{\overset{OAc}{C}}\text{——}\underset{H}{\overset{OAc}{C}}\text{——}\underset{OAc}{\overset{H}{C}}\text{——}CH_2OTr$$

XLI



$$BrH_2C\text{—}\underset{OAc}{\overset{H}{C}}\text{——}\underset{H}{\overset{OAc}{C}}\text{——}\underset{H}{\overset{OAc}{C}}\text{——}\underset{OAc}{\overset{H}{C}}\text{——}CH_2Br$$

XLII

This reaction can probably be used with other primary trityl ethers by increasing the concentration of the hydrobromic acid, possibly by using liquid hydrogen bromide.

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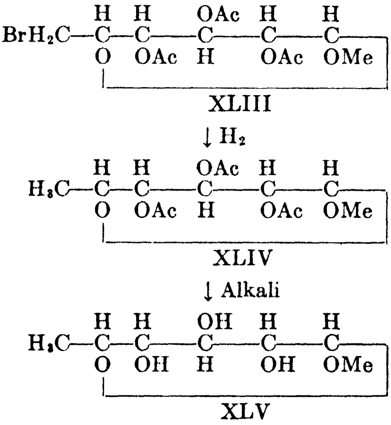
[79] A. Müller, *Ber.*, **65**, 1051 (1932).

[79a] M. L. Wolfrom, J. L. Quinn and C. C. Christman, *J. Am. Chem. Soc.*, **57**, 713 (1935).

The halodesoxy carbohydrates obtained from trityl ethers in this manner have been used in a number of chemical reactions, examples of which are given below.

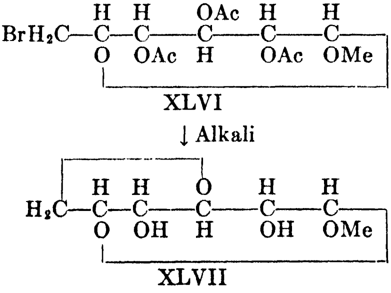
6. *Desoxy Sugars*

Replacement of the primary halogen atom of a terminally halogenated carbohydrate by a hydrogen atom leads to a desoxy sugar or methylose. This fact was established many years ago through the synthesis of isorhamnose.[80] However, some of these sugars became accessible only through the halodesoxy sugars prepared from trityl ethers. Thus methyl α-D-isorhamnoside (XLV) was prepared by the following series of reactions.[76]



7. *Anhydro Sugars*

Under certain conditions halodesoxy derivatives prepared from trityl ethers may be converted to anhydro sugars. Methyl α-D-glucopyranoside 3,6-anhydride[78] (XLVII) was prepared in this manner. The corresponding β-isomer had been described by Fischer and Zach.[80]



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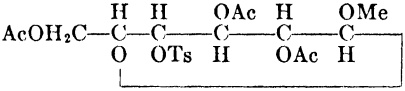
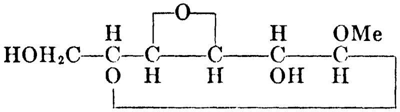
(80) E. Fischer and K. Zach, *Ber.*, **45**, 459 (1912).

The tosyl esters react like the halogen compounds. Methyl 4-tosyl-2,3,6-triacetyl-β-D-glucopyranoside (XLVIII), which may be obtained from methyl 6-trityl-β-D-glucopyranoside (see page 92), did not give a 4-tosyl derivative even on very careful deacetylation. The sulfonic acid residue was split off simultaneously and a peculiar anhydride[62] was formed. Müller[81] suggested a methyl 3,4-anhydro-D-hexoside structure (XLIX) for this substance.



$$AcOH_2C-\underset{O}{\overset{H}{C}}-\underset{OTs}{\overset{H}{C}}-\underset{H}{\overset{OAc}{C}}-\underset{OAc}{\overset{H}{C}}-\underset{H}{\overset{OMe}{C}}-$$

XLVIII

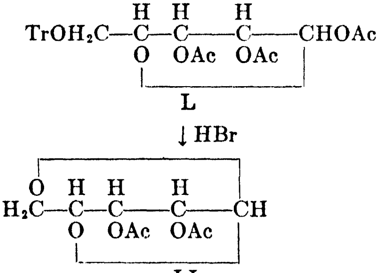


$$HOH_2C-\underset{O}{\overset{H}{C}}-\underset{H}{C}\overset{O}{\frown}\underset{H}{C}-\underset{OH}{\overset{H}{C}}-\underset{H}{\overset{OMe}{C}}-$$

XLIX

Micheel and Spruck[58] improved the synthesis of a pentaacetyl-D-galactoseptanose by using 6-trityl-2,3,4,5-tetraacetyl-D-galactose diethyl mercaptal as the starting material.

According to Bredereck and coworkers,[33] 5-trityl-1,2,3-triacetyl-D-ribofuranose (L) does not yield triacetyl-D-ribose on detritylation, but rather 2,3-diacetyl-D-ribofuranose 1,5-anhydride (LI).



$$TrOH_2C-\underset{O}{\overset{H}{C}}-\underset{OAc}{\overset{H}{C}}-\underset{OAc}{\overset{H}{C}}-CHOAc$$

L

↓ HBr

$$\underset{O}{\overset{O}{H_2C}}-\underset{O}{\overset{H}{C}}-\underset{OAc}{\overset{H}{C}}-\underset{OAc}{\overset{H}{C}}-CH$$

LI

8. *Unsaturated Carbohydrates*

Unsaturated carbohydrates may be prepared by the removal of hydrogen halide from certain halogenated carbohydrates which, in turn, are available from trityl ethers. Thus far this reaction has been successfully carried out only with compounds containing a primary halogen atom.

On attempting to transform the 6-bromo-6-desoxy or 6-iodo-6-desoxy derivatives of D-glucose into the corresponding 6-fluoro-6-desoxy compounds by means of silver fluoride in pyridine, the loss of hydrogen halide

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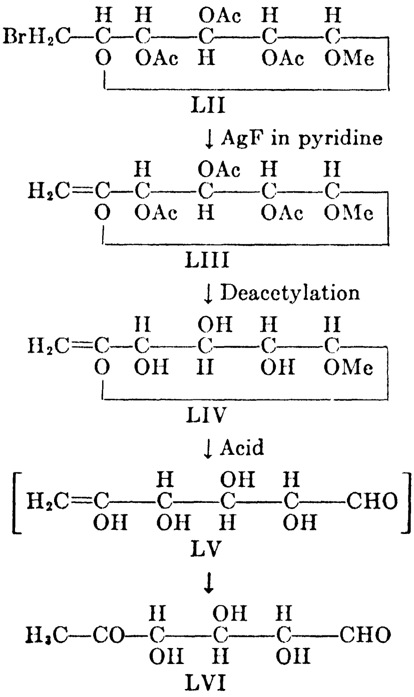
(81) A. Müller, *Ber.*, **67**, 421 (1934).

was observed. The resulting derivatives of D-glucose, unsaturated in the 5,6-position, were called glucoseens.[82,83,84] Thus, methyl 2,3,4-triacetyl-6-bromo-6-desoxy-α-D-glucopyranoside (LII) (or the corresponding 6-iodo compound) gave methyl 2,3,4-triacetyl-5,6-α-D-glucopyranoseenide (LIII). Acid saponification yielded the amorphous 5-ketoisorhamnose[83] (LVI), the first representative of a new class of dicarbonyl sugars. The C5 hydroxyl group of LV (formed after removing the glycosidic methyl group and opening the pyranose ring) is enolic and spontaneously rearranges to a keto group.



$$BrH_2C-\underset{O}{\overset{H}{C}}-\underset{OAc}{\overset{H}{C}}-\underset{H}{\overset{OAc}{C}}-\underset{OAc}{\overset{H}{C}}-\underset{OMe}{\overset{H}{C}}$$

LII

↓ AgF in pyridine

$$H_2C=\underset{O}{C}-\underset{OAc}{\overset{H}{C}}-\underset{H}{\overset{OAc}{C}}-\underset{OAc}{\overset{H}{C}}-\underset{OMe}{\overset{H}{C}}$$

LIII

↓ Deacetylation

$$H_2C=\underset{O}{C}-\underset{OH}{\overset{H}{C}}-\underset{H}{\overset{OH}{C}}-\underset{OH}{\overset{H}{C}}-\underset{OMe}{\overset{H}{C}}$$

LIV

↓ Acid

$$\left[H_2C=\underset{OH}{C}-\underset{OH}{\overset{H}{C}}-\underset{H}{\overset{OH}{C}}-\underset{OH}{\overset{H}{C}}-CHO\right]$$

LV

↓

$$H_3C-CO-\underset{OH}{\overset{H}{C}}-\underset{H}{\overset{OH}{C}}-\underset{OH}{\overset{H}{C}}-CHO$$

LVI

V. Detection of Pyranose and Furanose Rings

The elimination of hydrogen halide from certain halogenated sugars may be used as a test for the size of the sugar's lactol ring.[82] Up to the present time it has been observed only when the carbon atom adjacent to the halogenated carbon atom (C5 in the case of LII) is involved in lactol ring formation. Thus, compounds such as 3,5-diacetyl-1,2-iso-

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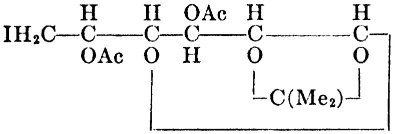
(82) B. Helferich and E. Himmen, *Ber.*, **61**, 166 (1928).

(83) B. Helferich and E. Himmen, *Ber.*, **62**, 2136 (1929).

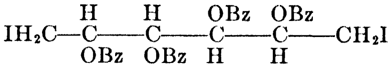
(84) B. Helferich and N. M. Bigelow, *Z. physiol. Chem.*, **200**, 263 (1931).

propylidene-6-iodo-6-desoxy-D-glucofuranose[85] (LVII) and 1,6-diiodo-1,6-didesoxy-2,3,4,5-tetrabenzoyl-D-mannitol[79] (LVIII) do not split off hydrogen halide or form unsaturated compounds under the influence of silver fluoride in pyridine.



$$IH_2C-\underset{OAc}{\overset{H}{C}}-\underset{O}{\overset{H}{C}}-\underset{H}{\overset{OAc}{C}}-\underset{O}{\overset{H}{C}}-\underset{O}{\overset{H}{C}}-$$

LVII



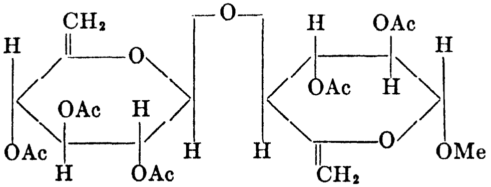
$$IH_2C-\underset{OBz}{\overset{H}{C}}-\underset{OBz}{\overset{H}{C}}-\underset{H}{\overset{OBz}{C}}-\underset{H}{\overset{OBz}{C}}-CH_2I$$

LVIII

If the halodesoxy sugar does not lose hydrogen halide when treated with silver fluoride in pyridine, it may be assumed that the carbon atom next to the halogenated one is not involved in the lactol ring; a positive reaction indicates that the carbon atom in question is thus involved.

If the loss of hydrogen halide did occur with compounds such as LVII or LVIII, the enol esters thus formed should show, after saponification, the reactions of hydroxyenols or hydroxyketones; for example, they should reduce Fehling solution. If the same lactol ring occurs in both the glycoside LII and the eenide LIV, and if the eenide does not reduce Fehling solution or show any other enol or ketone reactions, one of the doubly-bonded carbon atoms of the eenide must be a part of the lactol ring. This constitutes an independent proof for the position of the lactol ring, in the above case (LII), a pyranose ring.

By this method Müller[86] has confirmed the presence of a pyranose ring in methyl β-D-galactopyranoside. In the same manner, the pyranose rings were established in both D-glucose positions of methyl β-cellobioside by the preparation of a non-reducing methyl β-cellobiosedieenide (LIX).[60]

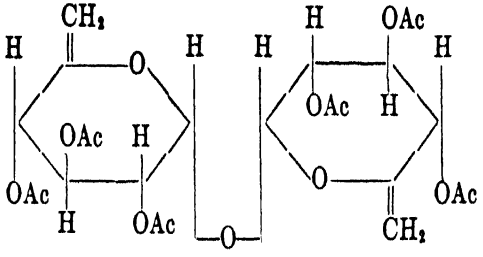


LIX

Bredereck[30] succeeded in preparing a hexaacetyltrehalosedieen (LX) from trehalose through ditrityltrehalose and 6,6′-diiodo-6,6′-didesoxytrehalose hexaacetate. Since this compound does not reduce Fehling solution after deacetylation and does not give other carbonyl reactions, both components of trehalose contain pyranose rings.

[85] B. Helferich and O. Lang, *J. prakt. Chem.*, [2] **132**, 321 (1932).

[86] A. Müller, *Ber.*, **64**, 1820 (1931).



LX

VI. Trityl Ethers of Glycerol and the Glycerides

Trityl ethers of polyhydroxy compounds have also been of importance in other fields of chemistry. Thus, for example, the synthesis of specific fats has been facilitated by the careful studies of Verkade and others[16,17,21,38,87–89] using trityl ethers. The first β-acylglycerol was that obtained from 1,3-ditritylglycerol.[90,91]

An elegant method has been developed by Fischer and Baer[92] for the transformation of D derivatives of glycerol into L derivatives with the aid of trityl ethers.

VII. Trityl Derivatives of Amines and Amides

The hydrogen atoms in amines and amides can also be replaced by trityl groups.[7] The N-trityl derivatives thus formed may prove of value in certain chemical reactions.

(87) P. E. Verkade, J. van der Lee and W. Meerburg, *Rec. trav. chim.*, **56**, 365 (1937).

(88) P. E. Verkade, J. van der Lee and W. Meerburg, *Rec. trav. chim.*, **56**, 613 (1937).

(89) D. T. Jackson and C. G. King, *J. Am. Chem. Soc.*, **55**, 678 (1933).

(90) B. Helferich and H. Sieber, *Z. physiol. Chem.*, **170**, 31 (1927).

(91) B. Helferich and H. Sieber, *Z. physiol. Chem.*, **175**, 311 (1928).

(92) H. O. L. Fischer and E. Baer, *Naturwissenschaften*, **25**, 588 (1937).

VIII. TABLE OF CARBOHYDRATE TRITYL ETHERS

Substance	*Melting point, °C.*	$[\alpha]_D^{20-25}$	*Rotation solvent*[f]	*References*
6-Trityl-α-D-glucopyranose (?)	60–100[a]	+59.6 → +38.0°	C_5H_5N	7
6-Trityl-1,2,3,4-tetraacetyl-α-D-glucopyranose	129–131	+97.8	C_5H_5N	7, 22
6-Trityl-1,2,3,4-tetraacetyl-β-D-glucopyranose	163–164	+45	C_5H_5N	7, 22
6-Trityl-3-benzyl-D-glucose	amorphous[a]	+45	C_5H_5N	49
6-Trityl-3-benzyl-1,2,4-triacetyl-D-glucopyranose	145–200[a]	+19.4	C_5H_5N	49, 93
Methyl 6-trityl-α-D-glucopyranoside	151–152	+86.3	C_5H_5N	27
Methyl 6-trityl-2,3,4-trimethyl-α-D-glucopyranoside	166–167	$+88.9_{5461}$	Me_2CO	27, 75
Methyl 6-trityl-2,3,4-triacetyl-α-D-glucopyranoside	136	+197	C_5H_5N	27, 76
Methyl 6-trityl-2,3,4-tribenzoyl-α-D-glucopyranoside	171 and 108–110[b]	+100	C_5H_5N	27
Methyl 6-trityl-2,4-diacetyl-3-tosyl-α-D-glucopyranoside	191–192	+72.8	$CHCl_3$	49
Methyl 6-trityl-β-D-glucopyranoside	108–109 and 148[b]	−37.6	C_5H_5N	27, 54
Methyl 6-trityl-2,3,4-triacetyl-β-D-glucopyranoside	126	+32	C_5H_5N	78
Methyl 6-trityl-2,3,4-tribenzoyl-β-D-glucopyranoside	99–101	—	—	94
Methyl 6-trityl-3-tosyl-β-D-glucopyranoside	76–78[c]	−22	$CHCl_3$	49
Methyl 6-trityl-2,4-diacetyl-3-tosyl-β-D-glucopyranoside	145–147	+14.5	$CHCl_3$	49
Methyl 6-trityl-2,3-dimethyl-3-tosyl-β-D-glucopyranoside	amorphous	−1.05	$CHCl_3$	49
Methyl 6-trityl-3-benzyl-2,4-dimethyl-D-glucopyranoside[d]	—	—	—	49
Phenyl 6-trityl-2,3,4-triacetyl-β-D-glucopyranoside	110–116	+16.9	$CHCl_3$	95
7′-(6-Trityl-β-D-glucopyranosyl)-theophylline	amorphous	+10.1	$CHCl_3$	50
7′-(6-Trityl-2,3,4-triacetyl-β-D-glucopyranosyl)-theophylline	135–145	+14.5	$CHCl_3$	50
6-Trityl-1,2-isopropylidene-3-acetyl-D-glucofuranose	amorphous	−16	$CHCl_3$	96

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[a] Impure. [b] Dimorphous. [c] Amorphous. [d] Anomeric mixture. [f] $c < 5$.

(93) K. Freudenberg and E. Plankenhorn, *Ann.*, **536,** 257 (1938).

(94) K. Josephson, *Ber.*, **62,** 313 (1929).

(95) B. Helferich and F. Strauss, *J. prakt. Chem.*, [2] **142,** 13 (1935).

(96) K. Josephson, *Ann.*, **472,** 217, 230 (1929).

VIII. TABLE OF CARBOHYDRATE TRITYL ETHERS (*Continued*)

Substance	*Melting point, °C.*	$[\alpha]_D^{20-25}$	*Rotation solvent*^{f}	*References*
6-Trityl-1,2-isopropylidene-3,5-diacetyl-D-glucofuranose	amorphous	+5	$CHCl_3$	96
6-Trityl-1,2-isopropylidene-3,5-dibenzoyl-D-glucofuranose	78–79 and 97–99^b	−4.5	$CHCl_3$	7
6-Trityl-D-glucopyranosyl fluoride	140	+58.4	C_5H_5N	64
6-Trityl-2,3,4-triacetyl-D-glucopyranosyl fluoride	147–148	+119.6	C_5H_5N	64
6-Trityl-2,3,4-tribenzoyl-D-glucopyranosyl fluoride	amorphous	+75.1	C_5H_5N	64
(6-Trityl-D-glucosyl)-trimethylammonium bromide	183–185	+14.8	EtOH	34
(6-Trityl-D-glucosyl)-pyridinium chloride	177	+42.4	EtOH	34
6-Trityl-2,3,4,5-tetrabenzoyl-D-glucose diethyl mercaptal	161–162	+49	$CHCl_3$	23
6-Trityl-1,2-ethylene-α-D-glucopyranoside	105–109	+37.0	$CHCl_3$	97
6-Trityl-1,2-ethylene-β-D-glucopyranoside	amorphous	+6.3	$CHCl_3$	97
Methyl 6-trityl-acetyl-β-D-glucopyranoside 3,4 (?)-anhydride	180.5–181.5	−91.8	$CHCl_3$	62, 81
Tris-(6-trityl-1,2-isopropylidene-D-glucofuranosyl) 3-phosphate	amorphous	−24.7	Me_2CO	36
3-Trityl-1,2:5,6-diisopropylidene-D-glucofuranose	115	−24.1	$CHCl_3$ and C_5H_5N	28
6-Trityl-D-mannose	140–170	−2.0	$CHCl_3$	29
		−3.7 → +20.4	C_5H_5N	
6-Trityl-1,2,3,4-tetraacetyl-α-D-mannopyranose	131–132	+73.4	$CHCl_3$	29
6-Trityl-1,2,3,4-tetraacetyl-β-D-mannopyranose	204–206	−2.6	$CHCl_3$	29
Methyl 6-trityl-α-D-mannopyranoside	101–102	+23.45	MeOH	56
Methyl 6-trityl-2,3,4-triacetyl-α-D-mannopyranoside	130	+44.3	$CHCl_3$	56
6-Trityl-2,3,4,5-tetraacetyl-D-mannose diethyl mercaptal	133.5–134.5	+36	$CHCl_3$	59
6-Trityl-2,3,4,5-tetrabenzoyl-D-mannose diethyl mercaptal	105–106	−10.5	$CHCl_3$	59
6-Trityl-D-galactose	72–75	+0.58 → +2.24	C_5H_5N	7
Methyl 6-trityl-β-D-galactopyranoside	167–168 (sinters at 80)	−39.48	$CHCl_3$	86

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(97) B. Helferich and Johanna Werner, *Ber.*, **75**, 1446 (1942).

VIII. Table of Carbohydrate Trityl Ethers (*Continued*)

Substance	*Melting point, °C.*	$[\alpha]_D^{20-25}$	*Rotation solvent*[f]	*References*
Methyl 6-trityl-2,3,4-triacetyl-β-D-galactopyranoside	138	−50.29	$CHCl_3$	86
Methyl 6-trityl-2,3,4-tribenzoyl-β-D-galactopyranoside	95	+77.75	$CHCl_3$	86
6-Trityl-1,2:3,4-diisopropylidene-D-galactopyranose	80–82	−58.4	$CHCl_3$	28
6-Trityl-2,3,4,5-tetraacetyl-D-galactose diethyl mercaptal	amorphous	−10.8	C_5H_5N	58
6-Trityl-2,3,4,5-tetrabenzoyl-D-galactose diethyl mercaptal	138–139	−22.5	$CHCl_3$	23
6-Trityl-2,3,4,5-tetraacetyl-*aldehydo*-D-galactose aldehydrol	152	−47 → −15	C_5H_5N	23, 58
6-Trityl-2,3,4,5-tetraacetyl-*aldehydo*-D-galactose ethyl hemiacetal	153	−28.4[e]	MeOH	23
6-Trityl-2,3,4,5-tetraacetyl-*aldehydo*-D-galactose semicarbazone	—	+20.0	$CHCl_3$	23
1-Trityl-D-fructose	170	−26 → +4.2	C_5H_5N	66
1-Trityl-2,3,4,5-tetraacetyl-D-fructose	146	+42.4	$CHCl_3$	66
1,6-Ditrityl-D-fructose·2 C_5H_5N	96–97	+17.5	$CHCl_3$	31
1,6-Ditrityl-D-fructose oxime	186	−18	C_5H_5N	31
1,2,6-Tritrityl-D-fructose	100–112[e]	+39.7	C_6H_6	15
1,2,6-Tritrityl-3,4-diacetyl-D-fructose	97–99[c]	+28	$CHCl_3$	15
1-Trityl-μ-mercapto-D-fructoxazoline	55–77[c]	—	—	98
1-Trityl-2,3:4,6-diisopropylidene-L-sorbofuranose	182	−27.5	$CHCl_3$	28
		−29.4	C_5H_5N	
1,2,6-Tritrityl-3,4-diacetyl-L-sorbofuranose	100–103[c]	+17.2	$CHCl_3$	15
Methyl trityl-α-L-fucopyranoside	123–126	−51.4	$CHCl_3$	42
Trityl-L-ascorbic acid	130[c]	+30	EtOH	99, 100

Mutarotates in methanol.

(98) G. Zemplén, A. Gerecs and Elizabeth Illes, *Ber.*, **71**, 590 (1938).

(99) L. v. Vargha, *Nature*, **131**, 363 (1933).

(100) P. Karrer, G. Schwarzenbach and K. Schöpp, *Helv. Chim. Acta*, **16**, 302 (1933).

VIII. TABLE OF CARBOHYDRATE TRITYL ETHERS (*Continued*)

Substance	*Melting point, °C.*	$[\alpha]_D^{20-25}$	*Rotation solvent*[f]	*References*
1-Trityl-D-xylose	80–98	—	—	15
1-Trityl-2,3-dibenzoyl-D-xylose	165	−4.87	C_5H_5N	15
1-Trityl-2,3-dibenzoyl-5-tosyl-D-xylose	171	+15.4	C_5H_5N	15
1,5-Ditrityl-D-xylose	80–100	+4.16	C_6H_6	15
1,5-Ditrityl-2,3-dibenzoyl-D-xylose	235	+31 (temp., 17°)	C_6H_6	15
Methyl ditrityl-β-D-xylopyranoside I	162.5–163	−22.5	C_5H_5N	41, 101
Methyl ditrityl-β-D-xylopyranoside II	238–240	−55.5	C_5H_5N	41, 101
Methyl trityl-diacetyl-β-D-xylopyranoside I	169–170	−15.7	$CHCl_3$	41, 101
Methyl trityl-diacetyl-β-D-xylopyranoside II	125–127	−49.1	$CHCl_3$	41, 101
5-Trityl-2,3,4-triacetyl-D-xylose diethyl mercaptal	149–150	−20.5	$CHCl_3$	23
5-Trityl-1-N-piperidyl-1-desoxy-D-threopentulose hydrochloride	148	−42.1	EtOH	15
5-Trityl-1-N-piperidyl-1-desoxy-D-threopentulose	68	−4.8	EtOH	15
1,5-Ditrityl-L-arabinose	86–93[c]	0	C_5H_5N	15
1,5-Ditrityl-diacetyl-L-arabinose	68–73[c]	—	—	15
1,5-Ditrityl-dibenzoyl-L-arabinose	210	—	—	15
5-Trityl-2,3,4-triacetyl-L-arabinose diethyl mercaptal	101–102	−24	$CHCl_3$	23
5-Trityl-2,3,4-tribenzoyl-L-arabinose diethyl mercaptal	111–112	−25	$CHCl_3$	59
5-Trityl-D-ribose	125	+12.1 → −9.9		33
5-Trityl-1,2,3-triacetyl-D-ribose	amorphous	+5.2	EtOH	33
1,5-Ditrityl-D-ribose	211	—	—	15
1,5-Ditrityl-2,3-diacetyl-D-ribose	285	—	—	15
1′-(5-Trityl-D-ribofuranosyl)-uracil	amorphous	+11.0	—	50, 51
1′-(5-Trityl-2,3-ditosyl-D-ribofuranosyl)-uracil	—	+27.7	Me_2CO	48
1′-(Ditrityl-D-ribofuranosyl)-uracil	223–224	—	—	48

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(101) E. L. Jackson, R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 947 (1934).

VIII. TABLE OF CARBOHYDRATE TRITYL ETHERS (*Continued*)

Substance	*Melting point, °C.*	$[\alpha]_D^{20-25}$	*Rotation solvent*[f]	*References*
1′-(5-Trityl-2,3-dimethyl-D-ribofuranosyl)-methyluracil	177–178	+96.1	Me_2CO	48
7′-(5-Trityl-D-ribofuranosyl)-adenine	259–260	−8.8	$CHCl_3$	50, 51
1′-(5-Trityl-D-ribofuranosyl)-cytosine	234–235	0	C_5H_5N	50, 51
7′-(5-Trityl-D-ribofuranosyl)-guanine	253–254	—	—	50, 51
6-Trityl-1,3:2,4-dimethylenesorbitol	194	−8.0	$CHCl_3$	102
1,6-Ditritylsorbitol	amorphous	−7.4	C_6H_6	19
1,6-Ditrityl-2,4-methylenesorbitol	106–108	—	—	102
1,6-Ditrityl-3,5-benzylidenesorbitol	100–103	+20	$CHCl_3$	39
1,6-Ditrityl-3,5-benzylidene-2,4-diacetylsorbitol	106–108	—	—	39
1-Trityl-2,3,4,5,6-pentaacetyl-D-mannitol	163–164	+35.5	$CHCl_3$	11
1,6-Ditrityl-D-mannitol	98–103[c]	−3.5	C_6H_6	19, 39
1,6-Ditrityl-tetraacetyl-D-mannitol	180–181	+46.4	$CHCl_3$	34
1,6-Ditrityl-tetrabenzoyl-D-mannitol	185	+46.9	$CHCl_3$	57
1,6-Ditrityldulcitol	183–184	—	—	11
1,6-Ditrityl-tetraacetyldulcitol	237–238	—	—	11
1,6-Ditrityl-dibenzylidenedulcitol	233–234	—	—	103
	240–242	—	—	11
1-Trityl-L-rhamnitol	132–135	+3.95	C_6H_6	19
1-Trityl-L-fucitol	142	−5.0	MeOH	19
1-Trityl-2,3,4,5-tetraacetyl-L-fucitol	152	−18	$CHCl_3$	11
1-Trityl-L-epirhamnitol (1-trityl-6-desoxy-L-glucitol)	68–72	−4.2	C_6H_6	19
1,7-Ditrityl-α-D-glucoheptitol (1,7-ditrityl-gluco-*gulo*-heptitol)	117–123	—	—	19

(102) E. J. Bourne and L. F. Wiggins, *J. Chem. Soc.*, 517 (1944).

(103) W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 139 (1942).

VIII. TABLE OF CARBOHYDRATE TRITYL ETHERS (*Continued*)

Substance	*Melting point, °C.*	$[\alpha]_D^{20-25}$	*Rotation solvent*[f]	*References*
1-Trityl-α-L-rhamnohexitol (1-trityl-7-desoxy-L-manno-L-*gala*-heptitol)	122–125	−20	MeOH-C_6H_6, 3:1	19
1,5-Ditritylxylitol	152–156	—	—	19
1,5-Ditrityl-2,3,4-triacetylxylitol	206	—	—	11
1,5-Ditrityl-L-arabitol	111–113	−3.5	C_6H_6	19
1,5-Ditritylribitol	141–145	—	—	19
1,4-Ditritylerythritol	182–184	—	—	19
6,6′-Ditrityltrehalose	278–281	+62.8	C_5H_5N	30
6,6′-Ditrityl-hexaacetyltrehalose	235–238	+115.7	$CHCl_3$	30
Methyl 6,6′-ditrityl-β-cellobioside	amorphous	−12.1	$CHCl_3$	60
Methyl 6,6′-ditrityl-pentaacetyl-β-cellobioside	amorphous	+19	$CHCl_3$	60
Tritrityltüranose	105–115[c]	+30.87	$CHCl_3$	104
Tritrityl-pentaacetylturanose	amorphous	+75.85	$CHCl_3$	104
Ditritylmaltose	137–139	+78	EtOH	96
Ditrityl-hexaacetylmaltose	116–119[c]	+91	$CHCl_3$	96
Tritritylsucrose	127–129[c]	+43.4	EtOH	96
Tritrityl-pentaacetylsucrose	125–126[c]	+57	$CHCl_3$	96
Tritritylraffinose	130[c]	+79	EtOH	96
Tritrityl-octaacetylraffinose	123–125[c]	+66	C_5H_5N	96
Tritylcellulose				20
Tritylstarch				20
Tritylglycogen				72

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(104) E. Pacsu, *J. Am. Chem. Soc.*, **53**, 3099 (1931).

GLUTOSE AND THE UNFERMENTABLE REDUCING SUBSTANCES IN CANE MOLASSES

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I. The Lobry de Bruyn-Alberda van Ekenstein Transformation

In 1897, Lobry de Bruyn and Alberda van Ekenstein[1] announced the preparation of two new ketohexoses, D-psicose* and glutose, and they suggested that the second had a carbonyl group on carbon 3. These observations, which were made in the course of their study of the action of weak alkali on simple sugars, although not supported by conclusive evidence, seemed entirely plausible because they appeared to be merely an extension of the work which had revealed that D-glucose, D-mannose and D-fructose were interconvertible in a weakly alkaline solution and that D-galactose yielded a mixture of D-tagatose and D-sorbose when treated with mild alkali. The latter two sugars were isolated in crystalline form.

Emil Fischer[2] had previously thought of the possibility of the existence of hexoses in unsaturated enolic forms, and on that basis Wohl and

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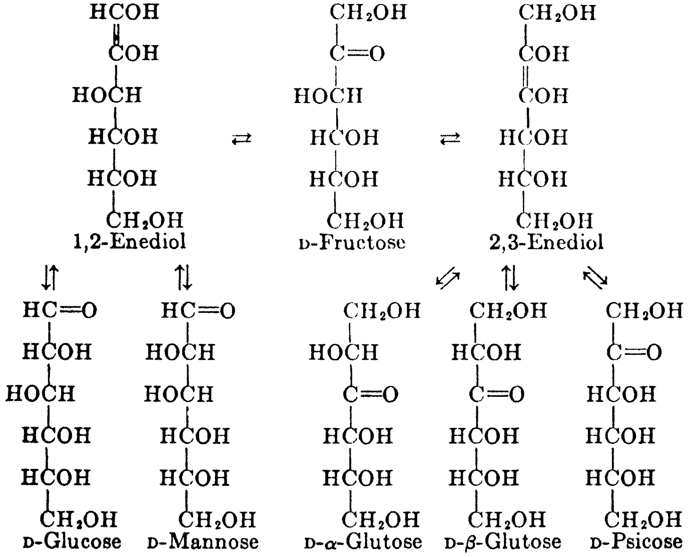
(1) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, (*a*) **16**, 257, 262, 284 (1897); (*b*) **18**, 72 (1899).

* The original name given by these workers was pseudofructose but various other names have been used for this sugar by subsequent investigators. H. Ohle and F. Just called it psicose, *Ber.*, **68**, 601 (1935); Marguerite Steiger and T. Reichstein used both names as well as D-ribo-2-ketohexose, *Helv. Chim. Acta*, **19**, 187 (1936); and W. L. Evans recommended the name allulose at the Atlantic City meeting of the American Chemical Society in September, 1941. Currently, M. L. Wolfrom, A. Thompson and E. F. Evans favor D-psicose, *J. Am. Chem. Soc.*, **67**, 1793 (1945), as a suitable and desirable trivial name and propose D-ribohexulose as a definite name. The name D-psicose will be used in this chapter.

(2) E. Fischer, *Ber.*, **28**, 1149 (1895).

Neuberg[3] proposed the theory that the equilibrium among D-glucose, D-mannose, and D-fructose predicated a 1,2-enediol which was the parent of all three. Nef[4] later pointed out that a 2,3-enediol could be the progenitor of two 3-ketohexoses, α-glutose, and β-glutose,† and a 2-ketohexose, D-psicose. Recently Wolfrom and coworkers[5] have furnished incontrovertible proof that a D-glucose 2,3-enediol does indeed exist by the finding of allitol (a reduction product of D-psicose) among the products obtained by the electroreduction of D-glucose at pH 10–13.

The various equilibria proposed by Nef[4] may be summarized as follows:



Lobry de Bruyn and Alberda van Ekenstein stated categorically that they had found glutose in cane molasses, and an analytical method for its quantitative estimation was published by them which was based on the nonfermentability of that sugar. That the unfermentable reducing

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(3) A. Wohl and C. Neuberg, *Ber.*, **33**, 3099 (1900).

(4) J. U. Nef, *Ann.* **357**, 296 (1907).

† Lobry de Bruyn and Alberda van Ekenstein erroneously considered various unrelated unfermentable reducing substances to be this same glutose. We shall reserve this name for the two 3-ketohexoses postulated in the equilibrium. In all other cases the term "glutose" (in quotation marks) will refer to other unfermentable reducing materials which have historical meaning only in the sense that they have been mistakenly associated from the beginning with the hypothetical 3-ketohexose, glutose.

(5) M. L. Wolfrom, B. W. Lew, and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 1443 (1946).

material in cane molasses and the "glutose" obtained by the action of lead hydroxide on D-fructose are identical was an unsubstantiated supposition. Sugar technologists have generally accepted the belief that D-glucose and D-fructose in cane juice are partially converted into D-mannose, D-psicose and glutose during the alkaline clarification process, but notwithstanding all efforts during the past fifty years, glutose itself has remained a mysteriously elusive substance which has defied isolation and characterization. While the over-all aspects of the Lobry de Bruyn-Alberda van Ekenstein transformation have stood the test of time, the work dealing with glutose has not fared so well, and there can also be some doubt regarding the identity of the material which they called pseudofructose. Prinsen-Geerligs[6] has reviewed the extensive literature dealing with the nature of the unfermentable reducing substances in molasses, but inasmuch as none of the papers arrived at any final elucidation, only those observations will be referred to which are germane to our present discussion.

Before going into a detailed study of the nature of "glutose," it will be helpful to quote (in translation) Lobry de Bruyn and Alberda van Ekenstein.[1a] We shall then have before us the original source of the misconceptions which have given body and substance to a mere inference and have misled subsequent workers.

> Ordinary glucose yields in addition to fructose and mannose a new ketose, pseudofructose, which has not yet been obtained in a pure state, and another substance, which is reducing but not fermentable, having the properties of a ketose and for which we suggest the name glutose. This substance can easily be prepared by the action of lead hydroxide on commercial fructose. . . . It can also be prepared by using invert sugar and lime.
>
> We wish to point out here that glutose plays a part in the ordinary sugar industry. Its presence in cane molasses, which we have proved, is to be explained on the same basis as that of mannose—namely, as the result of a transformation due to the action of lime. This explanation can clarify several observations made by chemists who have been or are still engaged in the study of sirups and of the undetermined losses which are known to occur in distilleries which use molasses. Despite numerous efforts repeated during a whole year, we have not succeeded in obtaining glutose in a crystalline state. The osazones which can be prepared from pseudofructose and from glutose are not identical with each other or with glucosazone. Glutose appears to bear the same relation to galtose as pseudofructose does to pseudotagatose.
>
> It will be observed that the hypothesis of intramolecular transformation advanced by us to explain the interconversion of glucose, fructose and mannose should be modified or rather extended. It would seem appropriate to postpone these theoretical considerations until the constitution of the new sugars has been established. It is to be noted, however, that the theory takes into consideration the existence of a great number of ketoses, it being possible for an intramolecular transformation of the kind indicated to take place among several carbons in the chain of the sugar. Fructose, for example, can produce in this way two new ketoses with a C=O on carbon 3, which can be transformed, by reduction, into four different hexitols, and yet can form one and the same osazone.

[6] H. C. Prinsen-Geerligs, *Intern. Sugar J.*, **40**, 345 (1938).

II. THE CHEMISTRY OF GLUTOSE

The actual chemical observations of Lobry de Bruyn and Alberda van Ekenstein can be summarized as follows. By the action of weak alkalies on D-glucose, they prepared "glutose" in the form of a sirup which would not crystallize, either directly or after solution in any solvent. This product gave the general reactions characteristic of ketoses—namely, Seliwanoff's test, complete destruction by prolonged boiling with dilute hydrochloric acid and ready formation of acids upon treatment with strong alkali. The substance differed from D-fructose by yielding a phenylosazone melting at 165°. They were unable to obtain D-glucose, D-fructose, and D-mannose by treating "glutose" with alkali in the same manner in which they prepared the supposed equilibrium mixture of hexoses from D-glucose and D-fructose. Furthermore, they found that "glutose" is formed simply by refluxing an aqueous solution of D-fructose in all-platinum equipment—that is, under conditions where no alkali is present and the solution soon turns acid.

In view of the fact that glutose is not present in an equilibrium mixture of isomers, it is evident that glutose cannot possibly be considered to be a product of a Lobry de Bruyn-Alberda van Ekenstein transformation, and its formation must be due to some other reaction. Lobry de Bruyn and Alberda van Ekenstein were struck and puzzled by the peculiar behavior of D-fructose. This sugar did not produce D-glucose when heated with a solution of lead hydroxide as it did when it was treated with potassium hydroxide, and a similar difference between the action of lead hydroxide and alkalies on other sugars was noted.[7] The complete conversion of D-fructose into glutose and acids was explicitly stated by Lobry de Bruyn and Alberda van Ekenstein not to be in accord with their supposition that the three sugars, D-glucose, D-mannose and D-fructose were interconvertible, and they wrote (in translation),[7b] "It is not improbable that our explanation of the intramolecular transposition of the sugars ought to be modified or extended." All this evidence makes it clear that Lobry de Bruyn and Alberda van Ekenstein were not committed to a 3-ketohexose structure for glutose, and that later workers were either so preoccupied with the validity of the generalized Lobry de Bruyn-Alberda van Ekenstein transformation that they accepted uncritically the original inference or they paid no attention to the contradictory evidence supplied by Lobry de Bruyn and Alberda van Ekenstein themselves.

Waterman and van der Ent[8] found that when cane juice is clarified at pH 8.0–9.5, unfermentable reducing substances are formed. The

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(7) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, (*a*) *Rec. trav. chim.*, **15**, 95 (1896); (*b*) *ibid.*, **16**, 275 (1897).

(8) H. I. Waterman and W. H. van der Ent, *Arch. Suikerind.*, **34**, II, 942 (1926).

same result was obtained with an artificial juice containing both D-glucose and D-fructose; when the D-fructose was omitted, no unfermentable reducing substances could be detected. Kilp[9] also reported that "glutose" was formed from D-fructose when cane juice was clarified, while Zerban and Sattler[10] obtained unfermentable reducing substances by heating invert sugar sirup at *p*H 4.5 or lower. If the Lobry de Bruyn-Alberda van Ekenstein transformation takes place under the conditions of cane juice clarification, then D-fructose should be formed from D-glucose in the experiments just mentioned, and this D-fructose should give rise to unfermentable reducing substances. It is possible that D-glucose alone, or any D-fructose formed from it, may have yielded too little unfermentable reducing substances to be detected by the method used; nevertheless, the experiments show that the D-fructose is the principal source. The conclusion is inescapable that alkalinity is not essential for the formation of unfermentable reducing substances from reducing sugars, and that the Lobry de Bruyn-Alberda van Ekenstein transformation can play only a minor part in the formation of unfermentable reducing substances during cane juice clarification. It was shown recently by Englis and Hanahan[11] that in pure aqueous solution, D-glucose can be autoclaved for thirty minutes at 15 pounds pressure without detectable change. However, in the presence of certain impurities such as potassium dihydrogen phosphate, or ammonium chloride, at *p*H 6.4–6.6, it is partially transformed into D-fructose, and at the same time "some ketose other than fructose, with dextro or less levorotation than fructose" is produced. Phosphates and other salts are always present in sugar cane products, and it is possible that under manufacturing conditions D-glucose is partially converted into D-fructose, but probably to a lesser extent than observed by Englis and Hanahan who employed a much higher temperature although the *p*H was approximately in the same range. The "ketose other than fructose" mentioned by them is evidently "glutose."

The first really definitive work on "glutose" was carried out by Benedict, Dakin and West.[12] They showed that "glutose" *in vitro* resembles D-fructose in that it is converted by sodium hydroxide into hydroxy acids (principally optically inactive lactic acid) to about the same extent as D-glucose. In slightly alkaline solution phenylhydrazine reacts to form the phenylosazone of methylglyoxal, and zinc ammonium hydroxide converts "glutose" into methylimidazole in yield comparable to that obtained from fermentable hexoses. But *in vivo* "glutose"

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(9) W. Kilp, *Z. Spiritusind.*, **55**, 188 (1932).
(10) F. W. Zerban and L. Sattler, *Ind. Eng. Chem.*, **34**, 1180 (1942).
(11) D. T. Englis and D. J. Hanahan, *J. Am. Chem. Soc.*, **67**, 51 (1945).
(12) Ethel M. Benedict, H. D. Dakin and R. West, *J. Biol. Chem.*, **68**, 1 (1926).

behaves entirely differently. Human tolerance is low, and in moderate doses it is excreted unchanged in the urine and by the bowel; the excretion of D-glucose by diabetics is not influenced by the administration of "glutose." Phlorizinized dogs excrete "glutose" quantitatively and no extra D-glucose is formed; ketosis is variably affected by "glutose"; the respiratory quotient in man is not changed after "glutose" consumption; insulin shock is not influenced by "glutose"; yeast, under conditions which are favorable for the formation of a hexose phosphate, produces no such ester from "glutose." Benedict, Dakin, and West concluded that "the inability of glutose to form a hexose phosphate is related to its non-fermentability by yeast, and its poor utilization by mammalian muscle."

Further insight into the nature of "glutose" was supplied by Spoehr and coworkers.[13] They found that when "glutose" is treated repeatedly with yeast, and the solution after each fermentation is heated to 80° for fifteen minutes, the copper reducing power diminishes. Of particular significance is their report that "glutose," by means of the cyanohydrin reaction, is convertible into a heptonic acid which upon reduction yields methyl-*n*-butylacetic acid. A 3-ketohexose would give ethyl-*n*-propylacetic acid. Only a 2-ketohexose behaves in this manner, and the "glutose" made by Spoehr and his collaborators was evidently a mixture of substances of unknown constitution, but which did not contain a 3-ketohexose.

A repetition of the work of Lobry de Bruyn and Alberda van Ekenstein by Coltof[14] showed that the unfermentable reducing substance obtained from simple sugars is not a new chemical individual but a mixture of compounds whose composition differs from that obtained from molasses. Although he could arrive at no definite conclusion from a study of the various osazones which he prepared, it was evident that the osazones were different depending on the source of the unfermentable residue. He could not accept the conclusions of Lobry de Bruyn and Alberda van Ekenstein regarding glutose, and instead offered the suggestion that the unfermentable substance contained dioses, saccharinic acids, glucic acid and albuminoids.

III. ANHYDRO-D-FRUCTOSE AND DI-D-FRUCTOSE ANHYDRIDE[15]

In a study whose inception had no bearing on the glutose problem, Mathews and Jackson[16] studied the decrease in rotation and reducing

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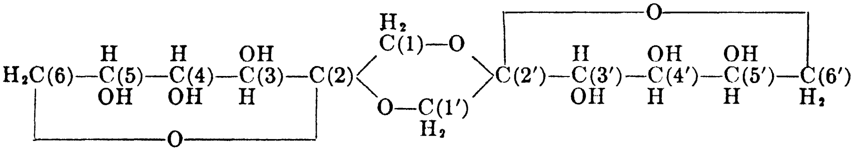
(13) (*a*) H. A. Spoehr and P. C. Wilbur, *J. Biol. Chem.*, **69**, 421 (1926); (*b*) H. A. Spoehr and H. H. Strain, *ibid.*, **85**, 365 (1929).

(14) W. Coltof, *Biochem. Z.*, **243**, 191 (1931).

(15) See Emma J. McDonald, *Advances in Carbohydrate Chem.*, **2**, 253 (1946).

(16) J. A. Mathews and R. F. Jackson, *Bur. Standards J. Research*, **11**, 619 (1933).

power that takes place when D-fructose solutions are heated under varying conditions of *p*H, temperature and time. They concluded from their experiments that the changes observed could be explained by the formation of D-glucose and D-mannose through the Lobry de Bruyn-Alberda van Ekenstein transformation and by a simultaneous formation of D-fructose anhydrides. The former reaction predominates at high *p*H, the latter at low *p*H, but there is no strict dividing line between the two. D-Fructose shows maximum stability at *p*H 3.3. Computations of the rotation values of D-fructose anhydrides in the reaction mixture indicated that they were probably identical with the amorphous heterolevulosan and the crystalline diheterolevulosan which Pictet and Chavan[17] obtained by treating D-fructose with concentrated hydrochloric acid at 0°. On the basis of analysis and molecular weight, Pictet and Chavan considered heterolevulosan to be a D-fructose anhydride, and diheterolevulosan a di-D-fructose anhydride, but they made only certain suggestions as to the chemical structures, based on analogies and unsupported by experimental evidence. Mathews and Jackson repeated Pictet and Chavan's work and confirmed their observations, but did not study the nature of the products. About the same time, Schlubach and Behre,[18] by treating D-fructose with liquid hydrogen chloride at room temperature, obtained a crystalline di-D-fructose anhydride which they showed to be a pyranose derivative, with a dioxane ring.



This compound may exist in at least three different modifications: α,α; α,β and β,β. Still further modifications are possible, depending on whether carbon 1 in one fructose unit is linked with carbon 1′ or with carbon 2′ in the other fructose unit.

Mathews and Jackson did not carry out fermentation experiments nor did they discuss the possible relation between D-fructose anhydrides and the "glutose" of Lobry de Bruyn and Alberda van Ekenstein. They merely concluded that the well-known reversion products in cane molasses probably consist of these same anhydrides. The term "reversion product" was first introduced by Wohl[19] to describe the sugar anhydrides formed when highly concentrated D-fructose or invert sugar solutions are heated in the presence of acid. He anticipated Mathews and Jackson's

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(17) A. Pictet and J. J. Chavan, *Helv. Chim. Acta*, **9**, 809 (1926).

(18) H. H. Schlubach and C. Behre, *Ann.*, **508**, 16 (1933).

(19) A. Wohl, *Ber.*, **23**, 2084 (1890).

observation that a minimum of reversion products is formed at a definite acidity, and he also found that at low sugar concentrations the reversion products are hydrolyzed by acid. This indicates an equilibrium between reversion and hydrolysis, depending on sugar concentration, acid concentration, time and temperature, provided no further decomposition of the primary reversion products has taken place. In addition, Wohl discovered that D-glucose is much more stable than D-fructose.

All of the work thus far described indicates that D-fructose anhydrides are intimately associated with the material called "glutose." Proof of this was supplied by Sattler and Zerban[20] who prepared an unfermentable sirup from D-fructose merely by refluxing an 80 per cent aqueous solution for sixteen hours. The water-free sirup obtained after fermentation was found to be soluble in anhydrous methanol but insoluble in acetone. It was possible to obtain a creamy-white, amorphous, very hygroscopic powder which reduced Fehling's solution, by pouring an absolute methanol solution of the sirup into a large excess of anhydrous acetone. Repetitions of this purification process with new acetone-precipitated material yielded increasing amounts of an alcohol-insoluble substance. This behavior recalls the observation of Pictet and Chavan[17] that heterolevulosan, which is soluble in alcohol, readily dimerizes into diheterolevulosan which is alcohol-insoluble. Mathews and Jackson[16] also concluded from their experiments that both the monomer and the dimer may be formed simultaneously. The decreasing reducing power of fermented and heated solutions of "glutose" previously noted, is thus understandable.

The specific rotation of one preparation of the amorphous powder was −28.3° (*c* 1.5, water) while that of another was −23.2° (*c* 0 3). The copper reducing power by the Munson and Walker method was 7.4 and 13.9%, respectively, of that of D-fructose. The molecular weight of the second preparation, determined by the freezing point method in water, was found to be 206, an indication that it contained about three parts of the monomer to one of the dimer.

If the unfermentable material consists of D-fructose anhydrides, as suggested by the work of Mathews and Jackson, its levorotation and reducing power should increase upon treatment with dilute acid due to a partial conversion of the product into D-fructose. When such a solution was heated to 60° with hydrochloric acid under conditions used in inverting sucrose according to the method of Jackson and Gillis[21] the specific rotation increased from −28.3° to −31.1°, or about 10%. When the acid solution was heated to 100° for one hour, the specific rotation rose to −65.6°. When the heating period was extended to four hours, the specific rotation dropped again to −24.9° because of decomposition. Similarly, hydrolysis according to the Jackson and Gillis method increased the reducing power from 7.4 to 14.3% of that of D-fructose; hydrolysis at 100° for four hours increased it to 28%. The original levorotation of the unfermentable material and its slow hydrolysis by acid are characteristic of the D-fructose anhydrides described by Pictet and Chavan, and by Schlubach and Behre.

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[20] L. Sattler and F. W. Zerban, *Ind. Eng. Chem.*, **37**, 1133 (1945).

[21] R. F. Jackson and Clara L. Gillis, *Bur. Standards, Sci. Papers*, No. **375**, 125 (1920).

Treatment of the unfermentable product with phenylhydrazine produced a phenylosazone which, when recrystallized from 50% alcohol, had a melting point of 194°. From it two fractions were obtained melting at 165° and 195°, respectively. The higher melting phenylosazone corresponded with a product melting at 194.5–195° obtained previously from the unfermentable residue of an invert sirup which had been kept at 55° for ten weeks.[10] Reindel and Frey[22] also reported a phenylosazone of the same melting point prepared from the unfermentable residue of cane molasses. The melting point of "glutosazone," according to Lobry de Bruyn and Alberda van Ekenstein is 165°. Nagel[23] found the same melting point for "glutosazone," but upon recrystallization it fell to 140°. Spoehr and Wilbur[13a] reported 163–166°, but in further work Spoehr and Strain[13b] observed that after each recrystallization "glutosazone" becomes more soluble and its melting point decreases. A fraction difficulty soluble in alcohol gave a melting point of 207°. Coltof[24] observed melting points ranging from 97° to 157° for the phenylosazone of various "glutose" preparations; the unfermentable residue from cane molasses yielded a phenylosazone melting at 163°. The extreme range of melting points reported for different "glutosazone" preparations demonstrates their nonhomogeneity.

It was found[20] that by simply washing the two phenylosazones melting at 165° and 195° with acetone, the melting points were raised to the correct melting point of D-glucose phenylosazone, namely 209–212°. Confirmation that the two so-called "glutosazones" were in fact impure D-glucose phenylosazones was obtained by converting crude "glutosazone" into phenyl-D-glucosotriazole by the method of Hann and Hudson.[25] Additional evidence was furnished by the observation that when "glutosazone" is first washed with acetone, and then recrystallized from dilute alcohol, the resulting phenylosazone, when mixed with authentic D-glucose

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(22) F. Reindel and A. Frey, *Z. Spiritusind.*, **57**, 237 (1934).

(23) J. A. R. Nagel, *Arch. Suikerind.*, **38**, III, 1139 (1930).

(24) W. Coltof, *Biochem. Z.*, **243**, 191 (1931).

(25) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 735 (1944).

phenylosazone, shows no change in melting point. The material soluble in acetone appears to be mainly the phenylosazone of methylglyoxal (pyruvic aldehyde).

On methylation of the unfermentable mixture,[20] a sirupy product was obtained which boiled at 216° at 1.1 mm., and reduced Fehling solution only slightly. However, when it was first hydrolyzed by prolonged boiling with hydrochloric acid, the resulting solution vigorously reduced Fehling solution. An analysis of the sirupy methylated product indicated that it was either trimethyl-D-fructose anhydride or else hexamethyl di-D-fructose anhydride. The molecular weight, however, was found to be 350–368, intermediate between that of the monomer (204) and the dimer (408); this result is in agreement with the observation that the reducing power increased upon hydrolysis and that D-fructose anhydrides are known to form dimers,[18] a property shared by their benzoylated and acetylated derivatives. In this connection it is of interest to call attention to the work of Schlubach and Elsner[26] which showed that their D-fructofuranose anhydride displayed the property of ready dimerization into a di-D-fructofuranose anhydride.

The specific rotation of the sirupy methylated product was +15.9° (*c* 3.0464, chloroform). Upon standing for five months at room temperature, a small quantity of crystals appeared which after purification melted at 96° and had a specific rotation of −41.9° (*c* 0.9500, chloroform). Schlubach and Behre[18] reported that their hexamethyldi-D-fructose anhydride had a melting point of 143–145°, and a specific rotation of −46.5°. These differences in physical constants may indicate the presence of different modifications as mentioned previously.

The same conclusion can be drawn from the physical properties of the hexaacetate of di-D-fructose anhydride, which has thus far been obtained only in the form of plastic sheets resembling glass. Table I compares the melting points and specific rotations of other di-D-fructose anhydride hexaacetates reported in the literature.

TABLE I

Physical Constants of Hexaacetyldi-D-fructose Anhydrides

Method of preparing anhydride	*M.p. of acetate, °C.*	$[\alpha]^{20}_D$ *(Benzene)*	*c*	*Reference*
D-Fructose and concd. HCl at 0°C.	94	−43.5°	—	17
D-Fructose and liquid HCl	171–73	−41.7	1.01	18
D-Fructose heated in water soln.	71	−44.4	0.896	20

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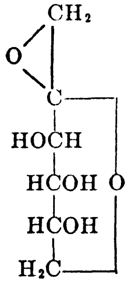
(26) H. H. Schlubach and H. Elsner, *Ber.*, **61**, 2358 (1928).

Interestingly enough, the hexabenzoate, although not crystalline, was very much like the compound previously described by Pictet and Chavan.[17] The specific rotation was −125° (*c* 1.5, benzene) and the melting point was 116°, whereas the compound in the earlier work melted at 118° and had a specific rotation of −122.5° in benzene. It is quite probable that the formation of isomeric derivatives of di-D-fructose anhydride stems from the fact that the anhydro sugar itself is a mixture of different isomers.

Proof of the location of the carbon atoms involved in the formation of the monomeric anhydro-D-fructose was obtained[20] by hydrolyzing the sirupy hexamethyldi-D-fructose anhydride. Phenylosazones were obtained which showed no loss of methyl groups from the trimethyl-D-fructose, while the rotation of sirupy trimethyl-D-fructose placed it in the pyranose family. This meant that hexamethyldi-D-fructose anhydride on hydrolysis yielded 3,4,5-trimethyl-D-fructose, and that the parent substance whose formula is given on page 119 was similar to the di-D-fructose anhydride described by Schlubach and Behre.[18]

The cyanohydrin experiment of Spoehr and Strain,[13b] cited earlier, now takes on added meaning. The dioxane ring of di-D-fructose anhydride is much too stable to be affected by the hydrogen cyanide, and it follows therefore that the action takes place with a monomeric anhydro-D-fructose. In order to yield methyl-*n*-butylacetic acid, the monomer in the mixture called "glutose" must be the D-fructose 1,2-anhydride.



IV. D-Psicose in "Glutose" Obtained from Simple Sugars

It will be recalled that Lobry de Bruyn and Alberda van Ekenstein claimed that pseudofructose (D-psicose) was one of the products formed as a result of a transformation of D-fructose and D-glucose in mildly alkaline solution. The doubt that one can have about its presence in the "glutose" mixture rests on their observation that D-psicose is fermentable. Synthetic D-psicose prepared by Steiger and Reichstein[27] is

(27) Marguerite Steiger and T. Reichstein, *Helv. Chim. Acta,* **19,** 187 (1936).

unfermentable, and Fischer and Baer[28] have shown that a mixture of D-fructose and D-sorbose synthesized from D-glyceraldehyde in the presence of $0.01M$ barium hydroxide does not contain either D-psicose or D-tagatose. D-Psicose in exceedingly small quantities was found by Zerban and Sattler[29] in distillery slop, but its presence there may have been natural and not due to the lime used in the manufacture of cane sugar.

V. Relationship between Nitrogen Content and Unfermentable Reducing Substances

The quantity of D-psicose in distillery residues obtained from cane molasses accounts for only a vanishingly small percentage of the total unfermentable material which is present. When concentrated distillery slop is first purified with neutral lead acetate, and then methylated, the chloroform extract yields relatively little of the hexamethylanhydro sugars derived from D-fructose and D-psicose, and the extract is nitrogen-free. Significantly enough, when cane molasses is passed through columns containing ion-exchange materials, there is no unfermentable reducing substance in the effluent.

A study[20] of the relationship between total nitrogen and unfermentable reducing substances, according to the procedure of the Java Sugar Experiment Station,[30] showed a striking correlation. Table II gives the results obtained from twelve final cane molasses of different origin; the molasses are arranged in the order of increasing nitrogen content.

The percentage of the unfermentable reducing substances increases in a general way with the total nitrogen. This is shown even better by the averages in Table II. It is evident that there is a good correlation between the nitrogen values and the unfermentable reducing constituents of molasses, although the molasses were produced in widely separated parts of the world. According to analyses by Browne,[31] 90% of the Kjeldahl nitrogen in cane juice, exclusive of that coagulated during clarification, is present in the form of amino acids and their amides (55% as amides, 35% as amino acids), 8% as soluble peptides, and 2% as ammonia. Hence practically all the Kjeldahl nitrogen in the clarified juice occurs in the form of groups which are capable of reacting with reducing sugars and undergoing the Maillard reaction.[32] Thus it may be expected that much of the nitrogen in molasses is present in the form

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(28) H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **19**, 519 (1936).

(29) F. W. Zerban and L. Sattler, *Ind. Eng. Chem.*, **34**, 1180 (1942).

(30) Proefstation Java-Suikerindustrie, "Methoden van Onderzoek," 6th ed., p. 365 (1931).

(31) C. A. Browne, *Louisiana Bull. Agr. Expt. Sta.*, No. **91**, 5 (1907).

(32) L.-C. Maillard, *Compt. rend.*, **154**, 66 (1911); *Ann. chim.*, [9] **5**, 258 (1916).

of nitrogenous condensation products, formed by the reaction of sugars and simpler carbonyl compounds with amino acids or their amides. Baars and Bogtstra[33] observed that the nitrogen in cane molasses is not utilized by the yeast *Torula utilis*, whereas nitrogen in the form of amino groups is readily assimilated.

TABLE II[20]

Total Nitrogen and Unfermentable Reducing Substances in Cane Molasses

Molasses Sample No.	*Total N, %*	*Unfermentable reducing substances expressed as invert sugar, %*
1	0.28	2.75
2	0.34	3.55
3	0.36	3.48
4	0.41	4.24
5	0.49	4.05
6	0.50	4.01
7	0.55	4.34
8	0.60	3.97
9	0.69	4.21
10	0.72	4.32
11	1.06	4.39
12	1.27	4.72
Ave. of first four values	0.35	3.51
Ave. of second four values	0.54	4.09
Ave. of third four values	0.94	4.41

The combined nitrogen in molasses was determined before fermentation and again, in the residue, after fermentation. Table III summarizes the results.

From 45% to 85%, or an average of 68% of the combined nitrogen in the original molasses is found in the unfermentable residue. Since it has not been assimilated by the yeast, it is not in the form of free amino groups, and the most probable explanation is that the amino groups have combined with the reducing sugars. The percentage of the total nitrogen present in the unfermentable portion is in the range reported by Ripp[34] for the percentage of total nitrogen, in the form of asparagine, that reacts with D-fructose (50.0–52.3%) and with D-glucose (80.8–85.1%). According to Browne[35] only 60% of the amino nitrogen in Louisiana cane juice is still found in the molasses; a Cuban molasses that

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(33) J. K. Baars and J. F. Bogtstra, *Arch. Suikerind. Nederland en Ned.-Indië*, **2**, 486 (1942).

(34) B. Ripp, *Z. Ver. deut. Zucker-Ind.*, **76**, 627 (1926).

(35) C. A. Browne, *Ind. Eng. Chem.*, **21**, 600 (1929).

had been stored for fifteen years contained no α-amino nitrogen whatever, as shown by a negative ninhydrin reaction.

D-Glucose and D-fructose, when heated in a phosphate-citric acid buffer solution of *p*H 7 in the presence of asparagine, produce considerable amounts of unfermentable reducing substances. D-Glucose, in the absence of asparagine, produces only a trace of unfermentable matter, while D-fructose alone produces a fairly large quantity—though considerably less than in the presence of asparagine. The reaction between D-glucose and asparagine yields the greatest amount of unfermentables.[20] Table IV shows these results.

TABLE III[20]

Nitrogen in Unfermentable Portion Compared with Total Nitrogen in Cane Molasses

Origin of molasses	*N as % of molasses*		*B as % of A*
	Total, A	*Unfermentable portion, B*	
Santo Domingo	0.37	0.22	60
Cuba	0.39	0.33	85
Cuba	0.44	0.21	48
Hawaii	0.45	0.20	45
Puerto Rico	0.50	0.42	84
Cuba	0.58	0.49	85
Florida	1.07	0.78	73

TABLE IV[20]

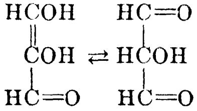
Reducing Power and Nitrogen Content of Unfermentable Condensation Products

	D-*Glucose alone*	D-*Glucose + asparagine*	D-*Fructose alone*	D-*Fructose + asparagine*
Cu, mg. from 50 ml.	0.7	154.8	25.9	101.3
Reducing substance, mg., calcd. as D-glucose or D-fructose, respectively	0.3	77.2	13.6	54.6
Reducing substance as % of sugar originally present	0.05	12.9	2.3	9.2
N, mg. in 20 ml.	0.0	21.3	1.4	39.6

Broadly speaking, two reactions are possible under these circumstances: anhydro sugar formation and condensation with active amino compounds. Although both of these reactions probably occur, it appears that the first type is favored. D-Glucose, which under comparable conditions shows very little tendency to form an anhydride, is able to produce a much greater amount of the condensation products than is D-fructose, which tends to form an anhydride much more readily.

Hydrolysis experiments[20] with artificially produced condensation compounds were carried out, and the curves relating the per cent increase in reducing power with the rate of hydrolysis of the unfermentable material were found to be similar to those obtained with the reducing substances derived from cane molasses.

Relatively small but appreciable quantities of reducing substances other than anhydro-D-fructose are found in heated D-fructose solutions which have been subjected to fermentation. Enders[36] has shown that hexoses produce traces of methylglyoxal when heated in aqueous solution. While methylglyoxal, as well as hydroxymethylfurfuraldehyde and levulinic acid, can be accounted for as primary products of thermal decomposition in acid solution, the by-products of fermentation are not to be neglected. Among these are acetylmethylcarbinol, identified by Browne[37] in fermented cane juice, diacetyl, methylglyoxal, and acetol. In addition, glucic acid (reductone)



$$\begin{array}{c}\text{HCOH}\\ \|\\ \text{COH}\\ |\\ \text{HC=O}\end{array} \rightleftarrows \begin{array}{c}\text{HC=O}\\ |\\ \text{HCOH}\\ |\\ \text{HC=O}\end{array}$$

is known to result from the action of alkali on D-glucose or D-fructose during juice clarification,[38] and the formation of more complex compounds from these and the nitrogenous compounds present in molasses is to be expected. In molasses, about 10% of the total amount of unfermentable reducing substances is volatile.

VI. Summary and Conclusions

The two 3-ketohexoses, which Lobry de Bruyn and Alberda van Ekenstein believed to be present in the unfermentable reducing material obtained on treatment of D-glucose or D-fructose with mild alkali, have not been found, and their existence under such conditions is doubtlul. The term "glutose," as applied to the unfermentable reducing substances obtained either from simple sugars or cane molasses, is ambiguous and misleading. The products which have been isolated from such unfermentable reducing substances include anhydro sugars and minor amounts of simpler organic compounds. The latter are formed as a result of thermal decomposition or fermentation and have structures which enable

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(36) C. Enders, *Wochschr. Brau.*, **60**, 98 (1943); *Biochem. Z.*, **312**, 339 (1942); *ibid.*, **312**, 349 (1942).

(37) C. A. Browne, *J. Am. Chem. Soc.*, **28**, 468 (1906).

(38) C. A. Browne, *J. Assoc. Official Agr. Chem.*, **30**, 394 (1937).

them to react with Fehling solution. In addition, all of these compounds are capable of reacting with amino acids or their derivatives (such as those present in molasses) thus giving rise to complex substances which are unfermentable but which behave as reducing sugars during analysis with Fehling solution. It follows that the quantitative methods for the estimation of "glutose" proposed by Lobry de Bruyn and Alberda van Ekenstein, and by Waterman and van der Ent, are unsound.

THE HALOGEN OXIDATION OF SIMPLE CARBOHYDRATES, EXCLUDING THE ACTION OF PERIODIC ACID

BY JOHN W. GREEN

The Institute of Paper Chemistry, Appleton, Wisconsin

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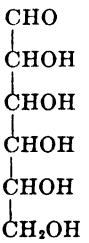
I. INTRODUCTION

The use of halogens for the oxidation of the carbohydrates is well known and the "bleaching effect" of halogens antedates fundamental work with the simple sugars. It is the purpose of this chapter to present an over-all picture of the work that has been done, with the hope that it will have an integrating effect and stimulate further work. The polysaccharides will not be considered here, because of the special problems involved and the lack of basic information. The work on periodic acid will also be omitted; an excellent review[1] of this oxidant for the whole field of organic chemistry has already been presented.

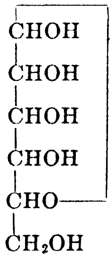
The action of the halogens on the simple sugars may be obscured for several reasons. The nature of some of the oxidants may be difficult to determine; the transient character of several of the oxyhalogen acids often serves to hide the real mechanism of the oxidation. In addition, it is often rather difficult to isolate the oxidation products of a sugar, especially when more than one product is produced in a reaction or when suitable analytical methods have not been developed.

The various types of carbohydrates considered in this chapter are shown in formulas I–XVIII. The two ring forms (II and III) of the aldoses are shown because careful oxidation can produce the lactones X and XI directly without breaking the oxygen bridge. In most cases, however, the products of oxidation have been isolated as salts of aldonic acids (IX); thus the nature of the initial oxidation product, either a lactone having the same ring structure as the sugar, or possibly the free

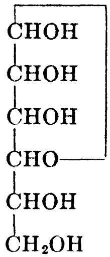


$$\begin{array}{l}\mathrm{CHO}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

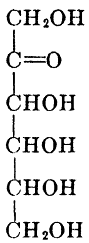
I
Aldose



II
Aldopyranose

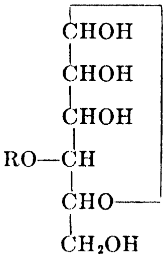


III
Aldofuranose

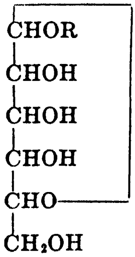


$$\begin{array}{l}\mathrm{CH_2OH}\\ |\\ \mathrm{C{=}O}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

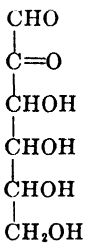
IV
Ketose



V
Disaccharide
(R = sugar unit)

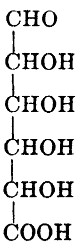


VI
Glyco-
pyranoside



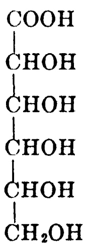
$$\begin{array}{l}\mathrm{CHO}\\ |\\ \mathrm{C{=}O}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

VII
Osone



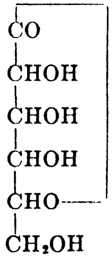
$$\begin{array}{l}\mathrm{CHO}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{COOH}\end{array}$$

VIII
Uronic
acid



$$\begin{array}{l}\mathrm{COOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

IX
Aldonic
acid



X
Aldonic 1,5-
lactone

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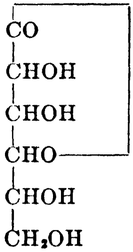
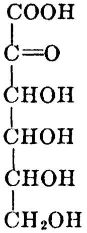
(1) E. L. Jackson *in* "Organic Reactions" (R. Adams, editor), Volume II, Chapter 8, John Wiley and Sons, New York (1944).

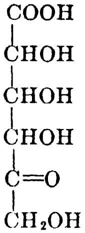


XI
Aldonic 1,4-lactone



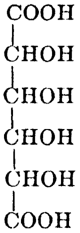
$$\begin{array}{l}\mathrm{COOH}\\ \vert\\ \mathrm{C{=}O}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CH_2OH}\end{array}$$

XII
2-Keto aldonic acid



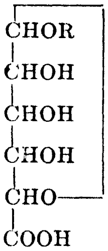
$$\begin{array}{l}\mathrm{COOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{C{=}O}\\ \vert\\ \mathrm{CH_2OH}\end{array}$$

XIII
5-Keto aldonic acid

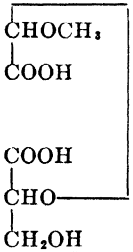


$$\begin{array}{l}\mathrm{COOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{COOH}\end{array}$$

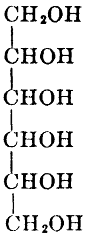
XIV
Saccharic acid



XV
Glycuronide

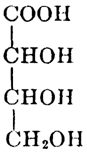


XVI
Methoxy-hydroxymethyl-diglycolic acid



$$\begin{array}{l}\mathrm{CH_2OH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CH_2OH}\end{array}$$

XVII
Hexitol



$$\begin{array}{l}\mathrm{COOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CHOH}\\ \vert\\ \mathrm{CH_2OH}\end{array}$$

XVIII
Trihydroxybutyric acid

acid derived from the aldehydo form I, is concealed. The preparation of aldonic acids from aldoses has formed the main part of the work in the field of halogen oxidation, both in the preparative and analytical fields.

Further oxidation of the aldonic acids results in the formation of either 2-keto or 5-keto aldonic acids (XII and XIII). After this step, degradation of the carbon chain apparently occurs. This degradation also occurs normally with ketoses (IV), a trihydroxybutyric acid (XVIII) being the main degradation product.

The oxidation of uronic acids (VIII) leads to the formation of saccharic acids (XIV); mild conditions are necessary to avoid damaging the sensitive uronic acid grouping. In a similar manner, osones (VII) may be converted to 2-keto aldonic acids (XII).

Oxidation of the primary alcohol group on C6 has been noted in a few cases. A small yield of saccharic acid (XIV) has been obtained in the formation of an aldonic acid from an aldose. In several cases glycosides have been oxidized to glycuronides (XV). More drastic action on the glycoside has led to a splitting of the carbon chain at C2—C3 and C3—C4, with elimination of C3, similar to the action of periodic acid; diglycolic acid derivatives (XVI) are formed. The oxidation of the primary alcohol group on C6 of ketoses has occasionally led to the formation of 5-keto acids (XIII).

The oxidation of reducing disaccharides (V) leads to the formation of aldobionic acids in a manner similar to the oxidation of aldoses to aldonic acids; conditions must be such that the glycosidic linkage is not hydrolyzed.

The great majority of these acids are isolated as the alkali or alkaline earth salts. The substituted hydrazides are also useful as intermediates. The lactones and free acids rarely crystallize readily unless fairly pure.

The polyhydric alcohols, ranging from glycerol to the hexitols (XVII), can also be oxidized, the products including both aldoses and ketoses. As the reaction mixtures are generally resolved as the phenylosazones, only a limited distinction between the products results. In the case of glycerol, D,L-glyceric acid and degradation products are also formed.

TABLE I

Oxidation Potentials of Various Halogen Oxidation Systems

Oxidation couple	*Solution*	*Calcd. potential, volts*[a,d]	*Solution pH*	*Observed potential, volts*[b,d]
Cl_2/Cl^-	Acid	1.36	1.9	1.35
$HOCl/Cl^-$	Neutral	1.49	6.1	1.19[c]
			7.5	1.14
OCl^-/Cl^-	Basic	0.94	10.0	0.92
Br_2/Br^-	Acid	1.07	2.0	1.12
$HOBr/Br^-$	Neutral	1.33	7.5	1.04
OBr^-/Br^-	Basic	0.76	10.0	0.98
I_2/I^-	Acid	0.53	2.2	0.55
HOI/I^-	Acid	0.99	—	—
OI^-/I^-	Basic	0.49	—	—
ClO_3^-/Cl^-	Acid	1.45	—	—
	Basic	0.62	—	—
BrO_3^-/Br^-	Acid	1.44	—	—
	Basic	0.61	—	—
IO_3^-/I^-	Acid	1.09	—	—
	Basic	0.26	—	—

[a] Calculated data; W. M. Latimer, "The Oxidative States of the Elements and their Potentials in Aqueous Solutions," Prentice-Hall Inc., New York (1938).

[b] Observed data; V. S. Bernstein, M. S. Dissertation, Institute of Paper Chemistry (1942).

[c] Observed data; L. J. Heidt, E. K. Gladding and C. B. Purves, *Paper Trade J.*, **121**, No. 9, 35 (1945).

[d] The potentials are expressed relative to the hydrogen electrode.

The oxidation potentials of various oxidation couples used in halogen oxidation are listed in Table I. These values give an idea of the relative strength of the various oxidants. In practice, however, quite different values are often found, due to the labile nature of the oxidant. The character of the latter is often affected by such factors as time, temperature, *p*H, concentration and light; one investigator listed twelve possible reactions for one system alone!

The question of halogen substitution has not arisen here. The earlier investigators attempted the isolation of halogenated sugars as possible intermediates in the oxidation reactions. None were found. In the case of some simpler alcohols and hydroxy aldehydes and ketones, substitution has been found to occur and the products have been hydrolyzed to "oxidation products." Since most of these halogenated compounds have been formed in non-aqueous systems, it seems doubtful that substitution would occur readily in the presence of water without decomposition of the halogenated products.

The recent developments in fluorine chemistry raise the possibility of using this halogen as an oxidant. Its action should be very much stronger than that of chlorine; elemental fluorine easily displaces oxygen from water. It may be difficult to control such a powerful oxidant in such a manner as to produce limited oxidation of organic compounds.

II. Oxidation of Simple Sugars with Halogens

The use of halogens as oxidizing agents for the simple sugars began in 1861 when Hlasiwetz[2] treated lactose with bromine water and obtained an aldonic acid as the product. Since then this field has undergone a great development, primarily concerned with the production of aldonic acids. Oxidation in strongly acidic solution has been developed as a preparative method, and has also been used to show the aldehydic structure of certain sugars for which a ketose structure was possible. The use of buffered weakly acidic solutions provided a means of oxidizing the ring forms of the sugars to the corresponding lactones. The application of alkaline hypoiodite solutions provided an oxidant that was approximately stoichiometrical in its reactions and would selectively oxidize aldoses in the presence of ketoses.

In spite of these advances, the mechanism of oxidation under various conditions is not entirely clear. Oxidation is often incomplete and it is frequently difficult to determine the nature and amount of oxidation product formed. Again the oxidizing system is such a complex mixture of halogen and oxyhalogen acids that it is very difficult to determine the actual oxidant and its concentration. Side reactions during the oxidation may change the very nature of the oxidant. It is, therefore, deemed advisable to present first a short review of the inorganic chemistry of the hypohalites and of the reactions that occur when halogens are treated

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(2) H. Hlasiwetz, *Ann.*, **119**, 281 (1861).

with water or aqueous alkali, with the hope that it will help the reader to become aware of some of the problems often encountered in this field.

1. *Inorganic Chemistry of the Hypohalites*

When bromine or chlorine is added to water, physical solution occurs followed by hydrolysis and formation of the corresponding hypohalous acid. In strongly acid solution

$$X_2 + H_2O \rightleftarrows HOX + HX \qquad (1)$$

the hypohalous acid concentration is very low. In the case of chlorine[3] it increases in the *p*H range 1–4 and is at its maximum in the *p*H range 3–6. As the alkaline side is approached (*p*H 5–10), the hypochlorous acid concentration decreases as the hypochlorite ion concentration increases; and

$$X_2 + 2NaOH \rightleftarrows NaOX + NaX + H_2O \qquad (2)$$

at *p*H 10 the concentration of hypochlorous acid is very small (see Table II).

Normally the equilibrium for reaction 1 is far to the left. The equilibrium constants[4] for the hydrolysis of chlorine, bromine and iodine are 4.5×10^{-4}, 2.4×10^{-8} and 3.6×10^{-13}, respectively.

However, the reaction can be shifted to the right by removal of the hydrogen halide; the hypohalous acids can thus be prepared by addition of compounds such as mercuric oxide, silver nitrate or silver phosphate[5]

TABLE II

Composition of Hypochlorite Solutions at Various Acidities[3]

*p*H	Cl_2, %	HOCl, %	OCl^-, %[a]
1	82	18	—
2	31	69	—
3	4	96	—
4	0.4	99.6	
5	—	(100)	—
6	—	96	(4)
7	—	73	27
8	—	21	79
9	—	3	97
10	—	0.3	99.7

[a] The original concentration of hypochlorite was 0.02 *M* and the temperature was 20–25°.

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(3) B. P. Ridge and A. H. Little, *J. Textile Inst.*, **33**, T33 (1942).

(4) J. W. Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. II, Longmans, Green and Co., London (1927).

(5) J. Ourisson and M. Kastner, *Congr. chim. ind., Compt. rend. 18me Cong.*, Nancy, Sept.–Oct. 1938, 938–991.

to the water-halogen mixture. The hypohalous acid is not strong enough to decompose the added salt and can be distilled directly from the mixture. A 5-6% solution of hypochlorous acid can be distilled without decomposition; by distillation under reduced pressure a 25% solution can be prepared.[5a] Since attempts to prepare stronger solutions lead to the formation of chlorine monoxide, the following equilibria must be involved:

$$HOCl \rightleftarrows H^+ + OCl^- \tag{3}$$
$$2HOCl \rightleftarrows Cl_2O + H_2O \tag{4}$$

Similarly, 1% solutions of hypobromous acid may be distilled at atmospheric pressure and 3% solutions may be prepared by distillation under reduced pressure at temperatures below 30°.[6] However, the hypobromous acid tends to decompose during the distillation, due to two simultaneous, but independent side reactions (5 and 6). In the dark at 15°, reaction 5 predominates[7]; it is independent of the *p*H.

$$5HOBr \rightarrow HBrO_3 + 2H_2O + 2Br_2 \tag{5}$$
$$4HOBr \rightarrow O_2 + 2H_2O + 2Br_2 \tag{6}$$

It is practically impossible to prepare a solution of hypobromous acid of known concentration, due to these reactions.

Solutions of hypoiodous acid cannot be distilled; they are very unstable and form iodic acid in a manner analogous to reaction 5 above. Their decomposition is accelerated by iodides and reducing agents.

The hypohalous acids are very weak and their salts may be decomposed by carbon dioxide. The respective dissociation constants of hypochlorous acid,[8] hypobromous acid[8,9b] and hypoiodous acid[9] are 6.5×10^{-8}, 2.0×10^{-11} and 5×10^{-13}. The values listed are representative ones; the data in the literature vary from 3.2 to 10.5×10^{-8} for hypochlorous acid, and the value for hypoiodous acid is only an estimate.

The structure of the hypohalous acids has received some study. Vorländer[9c] emphasized that the structure of hypochlorous acid must be H—O—Cl, not H—Cl—O, since the latter structure would indicate an acid as strong as hydrochloric acid. Thus, chlorine monoxide is con-

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(5*a*) It is interesting that two workers in the carbohydrate field, W. N. Haworth and J. C. Irvine, patented a preparation of hypochlorous acid [British Pat. 12,912 (1916)]. Chlorine gas was passed through a series of bottles of water containing a copper, cobalt or sodium salt or oxide as catalyst (140–170 g. per liter) or 10 g. of copper hypochlorite per liter. The product contained 2% hypochlorous acid.

(6) E. A. Shilov and N. P. Kanyaev, *J. Gen. Chem.* (*U. S. S. R.*), **8,** 445 (1938).

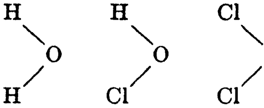
(7) F. Pollak and E. Doktor, *Z. anorg. allgem. Chem.*, **196,** 89 (1931).

(8) A. Skrabal and R. Skrabal, *Monatsh.*, **71,** 251 (1938); G. Holst, *Svensk Kem. Tid.*, **52,** 258 (1940).

(9*a*) A. Skrabal, *Ber.*, **75,** 1570 (1942); (*b*) *Z. Elektrochem.*, **48,** 314, 448 (1942).

(9*c*) D. Vorländer, *Ber.*, **34,** 1632 (1901).

sidered to be the anhydride of hypochlorous acid. Stieglitz[9d] pointed out that, for the formation of molecules of chlorine and water from hypochlorous acid (reaction 1), part of the acid must dissociate to produce Cl^+ and Cl^- ions. Hypochlorous acid must therefore dissociate not only as an acid, but also as a base. There is some evidence for the latter assumption.



$$HOCl \rightleftarrows H^+ + OCl^- \qquad (3)$$
$$HOCl \rightleftarrows Cl^+ + OH^- \qquad (7)$$

A solution of chlorine in water is essentially unstable but reaction 7*a*

$$2H_2O + 2Cl_2 \rightleftarrows 4HCl + O_2 \qquad (7a)$$

is reversible in light; even concentrated hydrochloric acid is oxidized in air to form a yellow solution. In dilute solution the decomposition of chlorine water is catalyzed by such salts as calcium chloride, ferric chloride, magnesium sulfate and manganese dioxide.[10] Decomposition in the dark at 25° is very slow. In contrast, hypochlorous acid is appreciably decomposed by sunlight[11]; the visible rays are very active while the ultraviolet rays have little effect. Sodium hypochlorite is attacked even more rapidly by visible rays than is hypochlorous acid.

The solubility of chlorine per 100 cc. of water at 20° is 1.85 g.; that of bromine is 3.58 g.; and that of iodine 0.28 g.[11a] Both chlorine and bromine form crystalline hydrates, $Cl_2 \cdot 8H_2O$ and $Br_2 \cdot 10H_2O$. They are stable only at low temperatures (0–9°). The increased solubility of bromine in potassium bromide solution is ascribed to the formation of KBr_3; if such solutions are saturated with bromine, the vapor pressure of the latter is the same as that of a water solution saturated with bromine. However, the halogen can be removed completely by extraction with carbon disulfide or by a stream of air; the KBr_3 must be stable only in the presence of free bromine. The solubility of iodine in water is increased by potassium iodide to 1.4 g. per 100 cc. and in ethanol a 20% solution can be formed.

The effect of alkali on the hydrolysis of halogens in water is to shift the equilibrium to the right in reaction 2 (page 134). In the case of chlorine, the reaction goes almost to completion. An excess of alkali

(9*d*) J. Stieglitz, *J. Am. Chem. Soc.*, **23**, 797 (1901).

(10) E. A. Shilov and G. V. Kupinskaya, *Byull. Ivniti*, **12**, no. 6, 39 (1936).

(11) L. Bonnet, *Rev. gén. mat. color.*, **39**, 29 (1935).

(11*a*) A. Seidell, "Solubilities of Inorganic and Metal Organic Compounds," Vol. I, D. Van Nostrand and Co., Inc., New York (1940).

prevents the hydrolysis of the hypochlorite salt (reaction 8) and even when chlorine equivalent to the alkali is added, the hydrolysis is small

$$NaOCl + H_2O \leftrightarrows HOCl + NaOH \qquad (8)$$

and a stable solution results. If an excess of chlorine is added, the hypochlorite will be hydrolyzed (reaction 9) and the resulting unstable hypo-

$$NaOCl + Cl_2 + H_2O \rightarrow NaCl + 2HOCl \qquad (9)$$

chlorous acid formed may then react with hypochlorite ion to form chlorate (reaction 10). The hydrochloric acid formed will react with

$$2HOCl + OCl^- \rightarrow 2HCl + ClO_3^- \qquad (10)$$

hypochlorite to form more hypochlorous acid and further the formation of chlorate.

A solution of hypochlorite is stable in an excess of alkali, whereas in acid there is a slow conversion to chlorate. The minimum stability of hypochlorite solutions is at pH 6.7[12] (6.68 and 7–7.13 have also been reported[13,14,14a]) and the maximum stability is at pH 13.[12] There is also a region of high stability in strong acid, where the solute is predominantly composed of chlorine and only very little hypochlorous acid. The rate of decomposition at pH 6.7–7.2 is proportional to the chloride concentration and to the square of the hypochlorite concentration.[15] In slightly alkaline solution the rate[16] is proportional to $[OCl^-][HOCl]^2$. The "catalytic" effect of chloride ion is well known; buffers[10] such as acetate, borate and carbonate also have an effect. It has been recommended that the concentration of phosphate buffers be kept below 0.25 M to prevent decomposition of hypochlorite solutions. The catalytic effect of heavy metals, especially copper and iron, has been noted. The effect of various cations is shown in the greater stability of lithium hypochlorite in comparison with the sodium and potassium salts.[14a]

In alkaline solution there is also a slow secondary change to chlorate, but this is not significant except at higher temperatures; it is accelerated by light and high hypochlorite concentration. The concentration effect is shown in Table III.

TABLE III

Effect of Alkali Concentration on Chlorate Formation[4]

NaOH in the original solution, %	8.5	12.0	27.3
$NaOCl/NaClO_3$	1/0.02	1/0.07	1/0.09

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(12) R. M. Chapin, *J. Am. Chem. Soc.*, **56**, 2211 (1934).

(13) J. M. Gallart, *Anales soc. españ. fís. quím.*, **31**, 422 (1933).

(14) K. M. Markuze, *Rekonstrukt siya Tekstil. Prom. Za.*, **14**, no. 5, 43 (1935).

(14*a*) P. Pierron, *Bull. soc. chim.*, [5] **10**, 445 (1943); *Compt. rend.*, **215**, 354 (1942).

(15) J. M. González Barredo, *Anales soc. españ. fís. quím.*, **37**, 220 (1941).

(16) J. M. Almedi y Iniquez, *Rev. univ. Zaragoza*, Jan.–Mar. (1936).

In the case of hypobromite solutions, the region of maximum stability[12] is at pH 13.4, a value slightly higher than that for hypochlorite solutions. Correspondingly, the region of minimum stability is slightly on the alkaline side, at pH 7.3. A region of stability on the strongly acid side has also been noted. The rate of decomposition of hypobromous acid at 25° and pH 6.4–7.8 has been determined[17]: $d[\mathrm{HOBr}]/dt = k[\mathrm{HOBr}]^3 [\mathrm{OH}^-] = k'[\mathrm{HOBr}]^2[\mathrm{OBr}^-]$.

The rate of decomposition of hypobromite solutions in the presence of excess alkali has been determined at various concentrations by Palmen.[18,19] In Table IIIa is shown the decreasing stability with increasing concentration of hypochlorite ions. This effect is similar to that shown for sodium hypochlorite (see Table III). The rate of decomposition is expressed as the "half-life."

Table IIIa

Rate of Decomposition of Hypobromite Solutions in the Presence of Excess Alkali

Cation	*Excess alkali*	*Temperature, °C.*	*Decomposition at various hypobromite concentrations* *Half life, hours* *0.5 N*	 *1.0 N*	 *2.0 N*
Li or K	10%	21–23	—	720	360
		34–36	—	96	48
		65–70	—	Very short	Very short
Na	50%	17–18	1272	552	240
		30–35	100	84	20
		60–65	6	5.5	0.8
		90–95	0.6	0.5	0.4

The formation of bromate in hypobromite solutions is about one hundred times as fast as that of chlorate in hypochlorite solutions and occurs readily in slightly alkaline solution, because of the greater hydrolysis of the sodium hypobromite. The velocity of iodate formation in hypoiodite solution proceeds at a much greater rate, about 3,000,000 times that for the chlorate. Hence, hypoiodite solutions are stable only in very low concentration or in the presence of a very small excess of alkali. An increase in temperature increases the rate of iodate formation. Ethanol will react readily with a cold solution of iodine in alkali with the

(17) C. F. Prutton and S. H. Maron, *J. Am. Chem. Soc.*, **57**, 1652 (1935).

(18) J. Palmen, *Finska Kemistsamfundets Medd.*, **43**, 151 (1934).

(19) J. Palmen, *Finska Kemistsamfundets Medd.*, **42**, 46 (1933).

formation of iodoform; if the alkaline solution is warmed before the addition of the ethanol, no iodoform will be formed.

The stability[20] of hypoiodite solutions at various alkalinities is shown in Table IV. The marked instability shown does not recommend the use of hypoiodite solutions for quantitative analysis when the oxidation goes at a rate slower than that for the decomposition of the oxidant.

TABLE IV

Hypoiodite Content of Iodine Solutions in Alkaline Media[20] *as Determined by Potentiometric Titration with 0.1 N Arsenite Solution*

Temperature, °C.	*Time, min.*	*Iodine solution*	*Alkali*	*Hypoiodite content*
0	0	5 cc. 0.1 *N*	7.5 cc. 0.1 *N* NaOH	100.00
	3	—	—	78.6
	4	—	—	72.2
	8	—	—	43.5
	12	—	—	33.8
	16	—	—	21.9
	30	—	—	16.3
	40	—	—	12.8
0	0	5 cc. 0.05 *N*	20 cc. Na_2CO_3—$NaHCO_3$ buffer at *p*H 10	100.00
	6	—	—	70.0
	10	—	—	57.1
	24	—	—	43.8
	40	—	—	39.6
	70	—	—	31.3
	120	—	—	21.7
	990	—	—	9.2
25	0	20 cc. 0.03 *N*	10 cc. 0.05 *M* borax buffer; *p*H 9.2	100.00
	10	—	—	98.6
	60	—	—	93.4
	150	—	—	86.9
	360	—	—	73.3
	1020	—	—	72.0

The only hypohalite salts known in the solid state are $NaOCl \cdot 5H_2O$ and bleaching powder. The structure of bleaching powder can be

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(20) E. Pacsu, *Textile Research J.*, **16**, 105 (1946).

expressed in several ways: $Ca(OCl)Cl$; $3Ca(OCl)Cl \cdot CaO \cdot 4H_2O$; $Ca(OCl)_2 \cdot CaCl_2 \cdot Ca(OH)_2 \cdot H_2O$. Bleaching powder undoubtedly owes its oxidizing action to the presence of the hypochlorite group. The decomposition with the liberation of oxygen is aided by the addition of cobalt salts. Carbon dioxide will cause hydrolysis; heat treatment causes dehydration and the explosive formation of chloride and chlorate.

Pure $NaOCl \cdot 5H_2O$ can be prepared[21] by the use of an alkyl ester intermediate. Chlorine is passed into a mixture of 30–35% sodium hydroxide solution and tertiary butanol. The alkyl hypochlorite is formed as a separate yellow layer; it is washed and then hydrolyzed with 40% sodium hydroxide solution until free of traces of heavy metals. The aqueous solution is cooled, and long pale yellow crystals of the pentahydrate are formed; they are stable at 0° for forty-eight hours.

2. *Halogen Oxidation in Strongly Acid Solution*

The oxidation of the aldehyde or hemiacetal group of an aldose sugar with bromine water leads to the formation of a strongly acid solution, regardless of whether the oxidant be considered as hypobromous acid or as free bromine; hydrobromic acid is formed in either case. Since the oxidation product is generally isolated from the reaction solution as the salt, very little can thus be learned about the formation of lactones directly from the ring forms of the sugars.

$$Br_2 + RCHO + H_2O \rightarrow RCOOH + 2HBr \qquad (11)$$
$$HOBr + RCHO \rightarrow RCOOH + HBr \qquad (12)$$

As previously mentioned, the first oxidation employing a halogen was carried out in 1861 by Hlasiwetz.[2] An aqueous solution of lactose was treated with an excess of bromine at 100° in a sealed tube. The acidic sirup obtained was termed "lactonic acid" and only the ammonium salt could be prepared in crystalline form. Barth and Hlasiwetz[22] succeeded in preparing other salts; treatment of the cadmium salt with hydrogen sulfide gave a crystalline product, $C_6H_{10}O_6$, the "lactonic acid," actually D-galactonic lactone. The free acid, $C_6H_{12}O_7$, was called a hydrate of the "lactonic acid." Kiliani[23] was the first to correct these workers and point out that hydrolysis of the lactose as well as oxidation had occurred and that both D-gluconic and D-galactonic acid could be expected as products.

The use of chlorine as an oxidant was investigated by Hlasiwetz and Habermann[24]; D-glucose was converted to D-gluconic acid and isolated

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(21) W. F. Underwood and E. Mack, Jr., *J. Phys. Chem.*, **35**, 2650 (1931).
(22) L. Barth and H. Hlasiwetz, *Ann.*, **122**, 96 (1862).
(23) H. Kiliani, *Ber.*, **18**, 1551 (1885).
(24) H. Hlasiwetz and J. Habermann, *Ann.*, **155**, 120 (1870).

as the barium and calcium salts. Treatment of the lead salt with hydrogen sulfide gave a sirupy acid which was obviously impure, as it reduced Fehling solution. This product was also obtained from sucrose. The oxidation of L-sorbose and D-fructose caused degradation and glycolic acid was isolated from the reaction products as the calcium salt.

a. Preparation of Aldonic Acids. A great deal of the work that followed centered on the oxidation of D-glucose and the isolation of D-gluconic acid, because of the interest in the use of calcium D-gluconate for intravenous injections to lessen surgical shock. Kiliani[25] carried out the bromine oxidation at 20°, instead of at the higher temperature used by Hlasiwetz. The reaction required several days and the calcium salt was isolated. The reaction seemed to involve only oxidation of the carbonyl group as no glycolic acid was isolated. A standard method was developed by Kiliani and Kleeman[26] and served as a model for the preparation of other aldonic acids. The maximum yields were 50–70% for the various acids. As noted later by Kiliani,[27] the oxidation does not go to completion and there is always unaltered aldose present under these conditions. The crystallization of the product is hindered by the presence of this starting material and, therefore, it is best to isolate the acid as a salt, either by crystallization from water or by precipitation from water into ethanol.

General Procedure of Kiliani and Kleeman[26] for the Preparation of Aldonic Acids. A cold solution of 1 part of D-glucose in 5 parts of water was shaken with 2 parts of bromine until all the latter had dissolved. Thirty-six hours were required for 100 g. of sugar. The solution was heated to remove the excess bromine and a bright yellow solution resulted. The latter was cooled, diluted to the original volume, and the amount of lead carbonate calculated for removal of bromide ions was added in small portions. The mixture was concentrated to one-half the volume and cooled for twenty-four hours; the lead bromide was filtered and washed with a minimum of cold water. The filtrate was diluted and treated with powdered silver oxide. The silver bromide was removed, and the filtrate treated with hydrogen sulfide to remove any silver present. The resulting solution was boiled with calcium carbonate, and the calcium D-gluconate solution concentrated to a small volume. The latter on cooling gave 70 g. of crude calcium D-gluconate. The salt was purified by recrystallization from water, or by precipitation from water into ethanol. An aqueous solution of the salt was treated with the calculated amount of oxalic acid; the calcium oxalate was removed and the filtrate was concentrated to a sirup, from which D-gluconic lactone gradually crystallized on drying under reduced pressure.

The above procedure was subject to various modifications. The bromine could be removed by aeration with air, carbon dioxide, hydrogen sulfide or sulfur dioxide. The hydrogen bromide formed in the reaction

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(25) H. Kiliani, *Ann.*, **205**, 182 (1880).
(26) H. Kiliani and S. Kleeman, *Ber.*, **17**, 1296 (1884).
(27) H. Kiliani, *Ber.*, **55**, 81 (1922).

was removed by silver oxide, silver carbonate or silver nitrate (the latter in the presence of nitric acid). Treatment with silver salts was generally followed by saturation with hydrogen sulfide to remove any silver ions in solution. Lead oxide or carbonate could be used in place of the silver compounds, or in conjunction with them; the cheaper lead salts were generally used first and then a small amount of silver salt added to remove the last traces of bromide. The lead ions were also removed by hydrogen sulfide. Barium carbonate was recommended by Kiliani[28]; the resulting barium D-gluconate was carefully precipitated into ethanol from a solution still containing bromide ions. If the ethanol concentration was kept at 30%, the barium bromide would not precipitate, and the salt of the acid was obtained directly.

In most cases, after the hydrogen bromide had been removed, the resulting solutions were treated with various carbonates or with ammonium hydroxide to form the desired salts. The salts could be recrystallized directly from water or precipitated into ethanol. The choice of salt depended on circumstances; thus, calcium D-gluconate crystallized well, but in the case of D-galactonic acid, the cadmium salt was preferable. The lead or cadmium salt was preferred when the free acid was desired since treatment of the aqueous solution or suspension with hydrogen sulfide easily removed the cation. The barium salts could be treated with the calculated amount of sulfuric acid and the calcium salts with oxalic acid for the same purpose.

The formation of barium, calcium or lead salts has one other purpose, that of eliminating any dicarboxylic acids as water-insoluble salts. Herzfeld[29] found that when zinc carbonate was used, the resulting zinc salts consisted of both D-gluconate and saccharate in the case of D-glucose; both were soluble in water and insoluble in ethanol, whereas in the case of the barium salts, only the D-gluconate was soluble in water.

Ruff[30] reduced the amount of bromine in the preparation of larger amounts of the aldonic acids, so that one part of bromine was used with one part of sugar. A yield of 75 g. of calcium D-gluconate was obtained from 100 g. of D-glucose and 49.5 g. of cadmium D-galactonate from 100 g. of lactose.

In a few cases the isolation of the free acid or lactone was carried out directly without the intermediate salt stage; Fischer and Herborn[31] isolated L-rhamnonic lactone by concentration of the aqueous solution after the bromide ions had been removed with lead carbonate, followed

[28] H. Kiliani, *Ber.*, **62**, 588 (1929).

[29] A. Herzfeld, *Ann.*, **220**, 335 (1883).

[30] O. Ruff, *Ber.*, **32**, 2273 (1899).

[31] E. Fischer and H. Herborn, *Ber.*, **29**, 1962 (1896).

by hydrogen sulfide and silver oxide. The product was recrystallized from hot acetone; an 80% yield of crude crystals was obtained.

As mentioned previously, the first preparation of D-galactonic acid was by the hydrolysis and oxidation of lactose. The cadmium salt isolated by Hlasiwetz was undoubtedly cadmium D-galactonate. Kiliani[23] noted that oxidation at 20° gave a very poor yield of this acid, probably because of incomplete hydrolysis; hence, the disaccharide was first hydrolyzed with sulfuric acid at 100°, then oxidized with bromine at 20°. A 70% yield (D-galactose basis) of cadmium D-galactonate was obtained. Later this process was modified[32] in that the oxidation was carried out at 20° with the disaccharide, after which the reaction mixture was heated at 100° for a few hours, then cooled and more bromine added at 20°. After the cadmium D-galactonate had been removed, the aqueous solution was treated with hydrogen sulfide and then with calcium carbonate, after which the calcium D-gluconate was isolated. Schnelle and Tollens[33] oxidized D-galactose and isolated the aldonic acid as the calcium salt. They noted that if an excess of bromine was used (2 parts instead of 1.3 parts per part of sugar) the yield of calcium D-galactonate was sharply decreased.

D-Mannonic acid was prepared by Fischer and Hirschberger[34]; since the calcium, barium and cadmium salts were all amorphous, the crystalline phenylhydrazide was isolated. Hydrolysis with barium hydroxide followed by removal of the phenylhydrazine with ether and the barium with sulfuric acid gave the crystalline lactone. The phenylhydrazide could also be obtained (15% yield) by the oxidation of hydrolyzed ivory-nut meal. D,L-Mannose was converted by Fischer[35] to D,L-mannonic acid and isolated as the phenylhydrazide.

D-Talonic acid was prepared by Braun and Bayer[36] and was isolated as the brucine salt, which was identical with that prepared earlier by Fischer.

The oxidation of L-rhamnose was carried out by Will and Peters[37] and by Rayman.[38] These workers isolated the lactone directly from the bromide-free solution without the intermediate salt stage. The product crystallized readily. This is one of the few instances where such a procedure can be carried out. As the calcium and barium salts were

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(32) H. Kiliani, *Ber.*, **59**, 1469 (1926).

(33) W. Schnelle and B. Tollens, *Ann.*, **271**, 81 (1892).

(34) E. Fischer and J. Hirschberger, *Ber.*, **22**, 3218 (1889).

(35) E. Fischer, *Ber.*, **23**, 370 (1890).

(36) J. von Braun and O. Bayer, *Ber.*, **58**, 2215 (1925).

(37) W. Will and C. Peters, *Ber.*, **21**, 1813 (1888).

(38) B. Rayman, *Ber.*, **21**, 2046 (1888).

amorphous, Schnelle and Tollens[39] isolated the strontium salt. Fischer and Herborn[40] obtained an excellent yield (80%) of the lactone directly from the water solution. Kiliani[27] reported maximum yields of 60–64% and noted that L-rhamnose reacted more slowly with bromine than did the other aldoses. The calcium L-rhamnonate was prepared as a stiff translucent jelly, drying to non-crystalline granules; the calcium salt was then converted by oxalic acid to the free acid.

The aldonic acids of the pentose series were prepared similarly. L-Arabonic acid was isolated by Kiliani[41] as the calcium salt, and Ruff[42,42a] prepared calcium D-arabonate. In the latter case almost quantitative yields were reported. D-Lyxonic acid was isolated as the basic lead salt and converted to the crystalline lactone by Wohl and List.[43] D-Ribose was oxidized to the crystalline cadmium D-ribonate by Levene and Jacobs[44]; the yield was less than 50%. The well-known "boat-shaped" crystals of the double salt of cadmium D-xylonate-cadmium bromide[45] were prepared by the oxidation of D-xylose with an excess of bromine by Ruff,[42a] followed by a subsequent treatment with cadmium carbonate. Ruff[42a] also prepared L-erythronic acid, isolating it as the brucine salt; the starting material was a crude L-erythrose sirup prepared by degradation of calcium L-arabonate.

Several of the desoxy aldoses have been converted to the corresponding aldonic acids. Mention has already been made of L-rhamnose. Bergmann, Schotte and Leschinsky[46] oxidized 2-desoxy-D-glucose to the crystalline barium 2-desoxy-D-gluconate; Fischer and Zach[47] obtained 6-desoxy-D-gluconic lactone (isorhamnonic lactone) from methyl β-D-isorhamnoside. Votoček[48] oxidized 6-desoxy-D-galactose to the lactone, and Müther and Tollens[49] obtained the enantiomorph from L-fucose (6-desoxy-L-galactose). 2-Desoxy-L-arabonic acid was obtained as the crystalline barium salt by Gehrke and Aichner[49a] from arabinodesose (2-desoxy-L-arabinose), and the lactone of 5-desoxy-L-arabonic acid was obtained from a sirupy 5-desoxypentose by Ruff.[50]

(39) W. Schnelle and B. Tollens, *Ann.*, **271**, 68 (1892).
(40) E. Fischer and H. Herborn, *Ber.*, **29**, 1962 (1896).
(41) H. Kiliani, *Ber.*, **19**, 3029 (1886).
(42) O. Ruff, *Ber.*, **32**, 550 (1899).
(42*a*) O. Ruff, *Ber.*, **34**, 1362 (1901).
(43) A. Wohl and E. List, *Ber.*, **30**, 3101 (1897).
(44) P. A. Levene and W. A. Jacobs, *Ber.*, **44**, 752 (1911).
(45) G. Bertrand, *Bull. soc. chim.*, [3] **5**, 554 (1891).
(46) M. Bergmann, H. Schotte and W. Leschinsky, *Ber.*, **56**, 1052 (1923).
(47) E. Fischer and K. Zach, *Ber.*, **45**, 3761 (1912).
(48) E. Votoček, *Z. Zuckerind. Böhmen*, **27**, 15 (1902).
(49) A. Müther and B. Tollens, *Ber.*, **37**, 306 (1904).
(49*a*) M. Gehrke and F. X. Aichner, *Ber.*, **60**, 918 (1927).
(50) O. Ruff, *Ber.*, **35**, 2360 (1902).

The oxidation of D-glucosamine to the 2-amino-D-gluconic acid is interesting.[51,52,53] The presence of an amino group adjacent to the carbonyl causes the oxidation with halogens to be very slow. The oxidation is readily effected by mercuric oxide.[53] Two to four weeks are required for oxidation by bromine and only 60% of the sugar is oxidized; yields of the acid range from 20 to 40%. Neuberg[52] noted that a longer oxidizing action of bromine was required for D-glucosamine hydrochloride than for the hydrobromide.

Chitose, a 2,5-anhydro-D-mannose obtained by Fischer[54] by the action of nitrous acid on D-glucosamine, was oxidized with bromine to chitonic acid (2,5-anhydro-D-mannonic acid).

The application of this method of oxidation to the disaccharides without accompanying hydrolysis was successful and proved to be a valuable structural tool in showing the nature of the linkage between the two sugar units. Lactose was converted to lactobionic acid (33% yield) by Fischer and Meyer[55]; the sugar was treated with an equal weight of bromine at 20° for two days. Several salts were prepared, notably the insoluble basic lead salt, but the free acid would not crystallize. Ruff and Ollendorf[56] reported a 38% yield, isolated as the calcium salt. Fischer and Meyer[57] also reported the preparation of maltobionic acid, as the basic lead or calcium salt; both were non-crystalline.

The value of a derivative such as lactobionic acid lies in the fact that it can be hydrolyzed to an aldose and an aldonic acid. Thus, the reducing portion of a disaccharide with two dissimilar sugar units can be identified. This has been applied to the trisaccharides as well. The trisaccharide rhamninose, obtained from xanthorhamnin, the glycoside of *Rhamnus infectoria*, can be oxidized by bromine to the corresponding aldonic acid. The rhamninotrionic acid[58] is hydrolyzed to two L-rhamnose units and one D-galactonic acid unit. In a similar manner, manninotriose (obtained from manna, the exudation of *Fraxinus ornus*) has been oxidized to manninotrionic acid and then hydrolyzed to D-galactose and D-gluconic acid.

Before attention is directed to the preparation of other oxidation products, the extensive use of bromine oxidation in the field of methylated sugars should be noted. The oxidation of the various sugars pre-

(51) E. Fischer and F. Tiemann, *Ber.*, **27**, 138 (1894).

(52) C. Neuberg, *Ber.*, **35**, 4009 (1902).

(53) H. Pringsheim and G. Ruschmann, *Ber.*, **48**, 680 (1915).

(54) E. Fischer, *Ann.*, **381**, 123 (1911).

(55) E. Fischer and J. Meyer, *Ber.*, **22**, 361 (1889).

(56) O. Ruff and G. Ollendorf, *Ber.*, **33**, 1806 (1900).

(57) E. Fischer and J. Meyer, *Ber.*, **22**, 1941 (1889).

(58) C. Tanret and G. Tanret, *Compt. rend.*, **129**, 725 (1899); C. Tanret, *ibid.*, **134**, 1586 (1902).

pared by Haworth and Hirst and their coworkers was utilized for the characterization of the methylated compounds. The work was initiated by Purdie and Irvine[59] who converted a tetramethyl-D-glucose to the lactone and barium salt, both non-crystalline. Purdie and Bridgett[60] converted a trimethyl-D-glucose to the lactone as evidence for the presence of an aldehyde group, since a satisfactory phenylhydrazone could not be prepared. Crystalline 2,3,4,6-tetramethyl-D-galactonic acid was obtained by Haworth, Ruell and Westgarth[61] by oxidation of the corresponding aldose with bromine at 15–35°. The acid crystallized from ether solution. Again, 2,3,4-trimethyl-D-xylonic lactone was obtained[62] in crystalline condition. Most of the methylated aldonic acids and lactones were obtained as sirups and were converted to the crystalline phenylhydrazides. The sirups could be distilled under reduced pressure. The preparation of 2,3,4-trimethyl-L-arabonic lactone was carried out[63] by a simultaneous hydrolysis and oxidation (hydrogen bromide and bromine) of the methyl glycoside; direct hydrolysis of the latter gave very poor yields because of the formation of furfural. The same procedure[62] was carried out with methyl 2,3-dimethyl-D-xyloside. The period of this work extended from 1924 to 1933 and embraced the derivatives of D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, D-lyxose and L-rhamnose. Both 1,4- and 1,5-lactones were prepared by the oxidation of the methylated aldoses.

b. Preparation of Products Other than Aldonic Acids. The oxidation of uronic acids with bromine represents the same type of reaction as that with the simple aldoses; the products are the saccharic acids. The advantage of the use of bromine water is that simultaneous hydrolysis and oxidation of uronides can be carried out, whereas direct hydrolysis of uronides might prove harmful to the sensitive uronic acid grouping. Thus, Lohmar, Dimler, Moore and Link[64] treated borneol D-glucuronide with *N* hydrogen bromide and bromine at 100° to obtain saccharic acid; pectic and alginic acids were treated similarly and mucic and D-mannosaccharic acids were obtained. Again, Wolfrom and Rice[65] treated barium acid heparinate with bromine and sulfuric acid and obtained D-glucosaccharic acid as proof of the presence of a D-glucuronic acid grouping. Heidelberger and Goebel[65a] showed the presence of a glucuronic acid unit in the "aldobionic acid" obtained by the hydrolysis of the specific polysaccharide of Type III pneumococcus. Refluxing with *N* hydrobromic acid and bromine gave saccharic acid as the reaction product, isolated as the potassium salt. A control with D-glucose gave no potassium acid saccharate.

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[59] T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1031 (1903).

[60] T. Purdie and R. C. Bridgett, *J. Chem. Soc.*, **83**, 1040 (1903).

[61] W. N. Haworth, D. A. Ruell and G. C. Westgarth, *J. Chem. Soc.*, **125**, 2473 (1924).

[62] W. N. Haworth and G. C. Westgarth, *J. Chem. Soc.*, 886 (1926).

[63] J. Pryde, E. L. Hirst and R. W. Humphreys, *J. Chem. Soc.*, **127**, 348 (1925).

[64] R. Lohmar, R. J. Dimler, S. Moore and K. P. Link, *J. Biol. Chem.*, **143**, 551 (1942).

[65] M. L. Wolfrom and F. A. H. Rice, *J. Am. Chem. Soc.*, **68**, 532 (1946).

Tiemann[66] studied the further oxidation of D-gluconic acid with bromine at 100°; a reducing sirup was formed which was isolated as an amorphous barium salt or a crystalline "osazone." Ruff[67] repeated the work and could find no "oxygluconic acid" in the oxidation mixture; most of the D-gluconic acid was unaltered and the reducing power was very low, especially in comparison with that obtained when keto aldonic acids were prepared by the action of hydrogen peroxide and ferrous salts. By ether extraction and distillation the formation of formic, oxalic and glycolic acids was shown.

The oxidation products of L-rhamnose treated with an excess of bromine for five days included a small amount of 5-keto-L-rhamnonic lactone; 1000 g. of L-rhamnose gave 550 g. of L-rhamnonic lactone and 55 g. of the keto derivative. In confirmation, Votoček and Malachata[68] achieved the partial conversion of L-rhamnonic lactone to the keto derivative. The very low yield, however, is indicative of the slowness of the reaction and the relative stability of the normal aldonic acids or lactones toward further oxidation.

Everett and coworkers have done extensive work on this "overoxidation" of aldoses with bromine at 25° for long periods of time (forty to fifty days). Under these conditions, appreciable amounts of the keto acids are obtained. Hart and Everett[69] obtained 5-keto aldonic acids from D-glucose, D-mannose, D-galactose, D-xylose and D-gulonic lactone. The barium salts were isolated and converted to the brucine salts. The structure of the oxidation product from D-xylose was not determined. The stability of these keto acids to further bromine oxidation had been noted earlier by Kiliani.[27]

One very interesting case is that of L-rhamnose. Hart and Everett obtained not only the expected 5-keto-L-rhamnonic acid but also 5-keto-L-mannonic acid (5-keto-D-gulonic acid); this was the first reported instance in which a $-CH_3$ group had been oxidized by bromine water to a $-CH_2OH$ group. The yields of the brucine salts of the two acids were

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(65a) M. Heidelberger and W. F. Goebel, *J. Biol. Chem.*, **74**, 613 (1927).

(66) W. Tiemann, *Z. Ver. Rübenzuckerind.*, **40**, 787 (1890).

(67) O. Ruff, *Ber.*, **32**, 2269 (1899).

(68) E. Votoček and S. Malachata, *Anales soc. españ. fis. quím.*, **27**, 494 (July–August 1929).

(69) J. P. Hart and M. R. Everett, *J. Am. Chem. Soc.*, **61**, 1822 (1939).

4.1 g. and 1.1 g. from 4 g. of L-rhamnose. The identity of the 5-keto-L-mannonic acid was checked by the fact that it was also prepared by the oxidation of D-gulonic lactone.

The yields of the 5-keto acids were not very high and the oxidation was not uniform. In the case of D-glucose, a 33% yield of the keto acid was obtained and 17% of the original sugar had been oxidized to the saccharic acid stage. Ten percent of other reducing material was present, and the remaining 40% of the product was believed to be D-gluconic acid, although this was not established.

Another method of preparing keto aldonic acids, those of the 2-keto type, is by oxidation of the corresponding osone. Neuberg and Kitasato[70] obtained 18 g. of calcium 2-keto-D-gluconate from 20 g. of the osone by the action of bromine at 20°. Similarly, 2-keto-D-galactonic and 2-keto-maltobionic acids were prepared by Kitasato.[71] The substances were characterized generally as the brucine salts because of the amorphous nature of the calcium salts.

Morrell and Crofts[72] have reported the degradation of D-glucosone by oxidation with bromine. A 25–30% yield of trihydroxybutyric acid was obtained. The experimental conditions here differed from those of Neuberg and Kitasato; less bromine was used for a shorter time at a higher temperature (40°). Since the yield of osone from the original D-glucose or D-fructose was very small, and the osone was prepared by the action of hydrogen peroxide rather than by conversion from the phenylosazone as in the work of Neuberg and Kitasato, the purity of the starting material in this work should be questioned. The osones are very difficult to obtain in any degree of purity, and the identification of a substance in solution by the formation of an osazone is inadequate. The formation of keto acids, as shown by Neuberg and Kitasato, rather than of trihydroxybutyric acid, is a better proof of the presence of an osone.

Herzfeld[73] reported a certain amount of oxidation on carbon atom six, as indicated by the formation of saccharic acid from D-glucose. The dicarboxylic acid was isolated from the normal oxidation mixture obtained in the preparation of D-gluconic acid from D-glucose or maltose. However, the reaction products were processed differently than usual; following the procedure of Grieshammer,[74] the acidic reaction mixture was neutralized with zinc carbonate instead of calcium or lead carbonate. Thus, the saccharic acid, which normally would be precipitated as an

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(70) C. Neuberg and T. Kitasato, *Biochem. Z.*, **183**, 485 (1927).

(71) T. Kitasato, *Biochem. Z.*, **207**, 217 (1929).

(72) R. S. Morrell and J. M. Crofts, *J. Chem. Soc.*, **81**, 666 (1902).

(73) A. Herzfeld, *Ann.*, **220**, 335 (1883).

(74) O. Grieshammer, *Arch. Pharm.*, [3] **15**, 193; *Brit. Chem. Abstracts*, **38**, 795 (1880).

insoluble salt, was converted into the soluble zinc salt. The mixture of zinc salts was precipitated into ethanol and then converted into the calcium salts. The latter were separated into two fractions; one was slightly soluble in water and was identified as calcium saccharate; the other was the normal calcium D-gluconate. Herzfeld did not mention specific yields of the products, except that the yield of calcium D-gluconate was less than when silver oxide was used to neutralize the acidic solution.

The well-known Ruff degradation of aldonic acids to aldoses with one less carbon was first applied with bromine as the oxidant. Calcium D-gluconate was treated with an excess of bromine at 20° for ten hours; the acidity of the solution was kept low with lead carbonate. The filtrate was processed and D-arabinose was obtained in small yield as the oxime. However, Ruff[75] found that the effect of hydrogen peroxide was much better and abandoned the use of bromine. Fenton[76] noted the same effect in the oxidation of tartaric acid to dihydroxymaleic acid; the action of oxygen was more effective than that of the halogens. It was assumed that a keto aldonic acid was the intermediate in the degradation of the aldonic acid to the new aldose, and the apparent stability of the keto acids to further oxidation by bromine may be the reason for the low yields with this oxidant.

c. Oxidation of Ketoses. The first oxidation of D-fructose was carried out by Kiliani.[77] Inulin was treated with boiling water and bromine; the reaction mixture was then cooled to 20° and oxalic acid, bromoform and glycolic acid were isolated. Herzfeld and coworkers[78] obtained both glycolic and trihydroxy-*n*-butyric acids, the latter in small amount. The reaction was very slow, taking several weeks. Hönig[79] obtained trihydroxy-*n*-butyric acid and also oxalic acid; the use of silver oxide was avoided, as it was felt that the formation of glycolic and formic acids arose from the action of this reagent, rather from that of the bromine. L-Sorbose behaved like D-fructose; Kiliani and Scheibler[80] noted that bromine did not attack either ketose in a period of eight days.

Hart, Sheppard and Everett[81] reported the production of 5-keto-L-gulonic and 5-keto-L-gluconic acids, respectively, from D-fructose and

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(75) O. Ruff, *Ber.*, **31**, 1573 (1898).

(76) H. J. H. Fenton, *J. Chem. Soc.*, **65**, 899 (1894); **69**, 557 (1896).

(77) H. Kiliani, *Ann.*, **205**, 182 (1880).

(78) A. Herzfeld and E. Bornstein, *Z. Ver. Rübenzuckerind.*, **23**, 42 (1886). A. Herzfeld and H. Winter, *ibid.*, **23**, 108 (1886).

(79) M. Hönig, *Ber.*, **19**, 171 (1886).

(80) H. Kiliani and C. Scheibler, *Ber.*, **21**, 3276 (1888).

(81) J. P. Hart, F. Sheppard and M. R. Everett, "Oxidation of Carbohydrates, Keturonic Acids and Salt Catalysis," page 9, Dept. of Biochemistry, Univ. of Oklahoma Medical School (1944).

L-sorbose by the action of bromine. The former product represented only one-third of the reducing material. The stability of the keto aldonic acids to bromine had previously been noted by several workers; Kiliani[82] prepared a keto-L-rhamnonic acid which did not react within four days with an excess of bromine. Similar results were reported by Everett, Edwards and Sheppard.[83]

Ruff[84] noticed in the mother liquors of an erythrose preparation obtained by degradation of L-arabonic acid, a small amount of another sugar, apparently a keto tetrose, which was not oxidized by bromine. The ketose gave the same phenylosazone as that obtained from L-erythrose, but was not isolated otherwise. Neuberg[85] oxidized erythritol to form an optically inactive tetrose solution and claimed that color reactions showed the presence of a keto tetrose.

d. Oxidation of Sugar Alcohols. Vincent and Delachanal[86] oxidized sorbitol with bromine in a sealed tube at 60°; the product was isolated as D-glucose phenylosazone. Fischer[87] confirmed this work and mentioned that a considerable amount of D-fructose was formed. D-Mannitol also gave D-fructose as an oxidation product. No data were given for the latter claim. The oxidation products from sorbitol, after being treated with phenylhydrazine, were fractionated with acetone, and both D-glucose phenylosazone and D-gulose phenylosazone were isolated by Votoček and Lukeš.[88] Thus, the possible formation of two aldoses and two ketoses was shown. Talen[89] repeated this work and removed the D-glucose and D-fructose by fermentation. As the unfermentable sugars gave only D-gulose phenylosazone, the presence of D-gulose and L-sorbose was indicated.

e. Chlorine Water as an Oxidant. The use of this oxidant has received very little attention. The early work of Hlasiwetz and Habermann[24] resulted in the formation of D-gluconic acid from D-glucose. Apparently yields were low, probably as a result of "over-oxidation," since this agent has a much higher oxidation potential than bromine water. Also chlorine water, even at a *p*H of 1 or 2, contains a high percentage (18–50%) of hypochlorous acid, which has an oxidation potential even higher than that of the chlorine-chloride couple. In contrast, bromine water

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(82) H. Kiliani, *Ber.*, **55**, 82 (1922).

(83) M. R. Everett, B. G. Edwards and F. Sheppard, *J. Biol. Chem.*, **104**, 11 (1934).

(84) O. Ruff, *Ber.*, **32**, 3675 (1899).

(85) C. Neuberg, *Z. physiol. Chem.*, **31**, 564 (1901).

(86) C. Vincent and Delachanal, *Compt. rend.*, **111**, 51 (1890).

(87) E. Fischer, *Ber.*, **23**, 3684 (1890).

(88) E. Votoček and R. Lukeš, *Rec. trav. chim.*, **44**, 345 (1925).

(89) H. W. Talen, *Rec. trav. chim.*, **44**, 891 (1925).

contains a very low concentration of hypobromous acid under any conditions. The reaction of chlorine with D-glucose is relatively slow, in comparison with that of bromine. Hlasiwetz and Habermann[90] found that the reaction took four to five days, whereas with bromine one to two days were required. No comparative study of the two oxidants has been reported; it may be that the concentration of the chlorine is maintained at a low level, thus accounting for the slow reaction.

Fletcher and Taylor[91] noted that chlorine water attacked D-gluconic acid rapidly with the formation of a reducing material. No attempt was made to isolate the substance.

f. Iodine as an Oxidant. Brunner and Chuard[92] concluded that iodine had no action on D-glucose. The sugar was treated with iodine and dilute ethanol in the presence of succinic acid at 110° for several hours; from the brown reaction mixture a monoiodosuccinic acid was obtained, but the D-glucose was apparently unaltered.

3. *Oxidation in Buffered Weakly Acid Solution*

The oxidation of aldoses with bromine water soon leads to the formation of a strongly acidic solution, owing to the formation of hydrobromic acid. It was recognized at an early date that the accumulation of this acid slowed up the oxidation, and Bunzel and Matthews[93] showed that the effect was not due solely to the increased acidity but that hydrochloric acid and hydrobromic acid had stronger inhibiting effects than other acids. Hönig and Ruzicka[94] studied the time required for the various sugars to react with an equal weight of bromine in water in the presence and in the absence of calcium carbonate. The reaction times for D-glucose were 5 and 150 minutes, respectively, and for D-fructose the times were 2 and 350 hours, respectively. The removal of the hydrobromic acid as it was formed had a very definite accelerating effect on the reaction.

The first attempt to maintain a low acidity was carried out by Clowes and Tollens[95] in 1899. A mixture of D-galactose, water and calcium carbonate was shaken with an excess of bromine for twelve to twenty-four hours. Carbon dioxide was evolved and the solution was concentrated at 100°. A thin sirup was obtained, from which calcium D-galactonate crystallized upon the addition of ethanol. The same procedure was

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(90) H. Hlasiwetz and J. Habermann, *Ber.*, **3**, 486 (1870).
(91) H. H. Fletcher and T. C. Taylor, *J. Am. Chem. Soc.*, **60**, 3023 (1938).
(92) H. Brunner and E. Chuard, *Ber.*, **19**, 595 (1886).
(93) H. H. Bunzel and A. P. Matthews, *J. Am. Chem. Soc.*, **31**, 464 (1909).
(94) M. Hönig and W. Ruzicka, *Ber.*, **63**, 1648 (1930).
(95) H. A. Clowes and B. Tollens, *Ann.*, **310**, 164 (1899).

applied to the preparation of calcium D-mannonate. No mention was made of the yield.

A 60% yield was reported by Ruff[96] for the preparation of L-arabonic acid when the oxidation with bromine was carried out in the presence of calcium carbonate; when the reaction was carried out with bromine alone, almost quantitative yields were obtained.

Glattfeld and Hanke[97] applied this procedure to the oxidation of maltose hydrate. An aqueous solution of the sugar was shaken with lead carbonate and bromine; since lead bromide separated from the reaction mixture, only aeration was necessary to remove the excess bromine, and treatment with silver oxide and hydrogen sulfide removed the last bromide, silver and lead ions. The maltobionic acid was isolated as the calcium salt. Here the neutralization of the hydrobromic acid was beneficial in preventing any hydrolysis of the aldobionic acid.

Ling and Nanji[98] pointed out the effect of the accumulated hydrobromic acid in slowing up the oxidation. In an industrial process for making D-gluconic acid, they used calcium carbonate to maintain a weakly acidic solution and claimed a 90% yield of calcium D-gluconate. The low acidity not only accelerated the reaction but also increased the yield. This method was indirect in that a stream of chlorine was passed into a sugar solution containing calcium bromide; the latter behaved as a catalyst, bromine being constantly formed. This action is similar to the electrolytic oxidation of Isbell and Frush (page 154) or the alkaline oxidation patented by Chemische Fabrik vorm. Sandoz (page 162).

Hudson and Isbell[99] developed the buffering action further. Calcium or barium benzoate was employed to maintain the oxidation solution at a *p*H of 5–6, and the reaction mixture was kept in the dark. Lactose was converted to lactobionic acid without hydrolysis, and 96 and 90% yields of calcium D-gluconate and cadmium D-xylonate-cadmium bromide were reported by the use of this method. Isbell and Pigman[100] developed this method later into a valuable tool for the study of the mechanism of bromine oxidation and for the study of the structure of various aldoses (see pages 171, 182).

Preparation of Calcium D-*Gluconate, according to Hudson and Isbell.*[99] Six cc. of bromine (0.12 moles) was added to an ice-cold solution of 18 g. (0.1 mole) of anhydrous D-glucose and 60 g. (0.15 mole) of barium benzoate in 750 cc. of water. The bromine

(96) O. Ruff, *Ber.*, **34**, 1362 (1901).

(97) J. W. E. Glattfeld and M. T. Hanke, *J. Am. Chem. Soc.*, **40**, 989 (1918).

(98) A. R. Ling and D. R. Nanji, *J. Soc. Chem. Ind.*, **41**, T28 (1922).

(99) C. S. Hudson and H. S. Isbell, *J. Am. Chem. Soc.*, **51**, 2225 (1929); *J. Research Natl. Bur. Standards*, **3**, 57 (1929).

(100*a*) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **10**, 337 (1933); (*b*) *ibid.*, **18**, 141 (1937).

dissolved rapidly when the solution was shaken and crystals of benzoic acid soon formed. Although a faint but definite Fehling test for reducing substances was obtained when light was not excluded, a negative test was obtained when the mixture was maintained for thirty-six hours at 20–25° in darkness. The excess bromine was removed by a stream of air and the barium was precipitated quantitatively with sulfuric acid; about 60 cc. of 5 *N* acid was used. The barium sulfate was removed, after the addition of about 10 g. of activated carbon. The hydrobromic acid was removed quantitatively by the use of lead carbonate (27 g.) followed by 5 g. of silver benzoate. If 28 g. of silver carbonate was used alone, a faster removal could be effected. The excess silver and lead ions were removed as sulfides and the filtrate was concentrated to 200 cc. under reduced pressure. Several extractions with chloroform removed any benzoic acid present. The resulting aqueous solution of D-gluconic acid was colorless and did not reduce Fehling solution.

The solution was neutralized with calcium carbonate and concentrated to 75 cc. The addition of 10 volumes of 95% ethanol precipitated granular calcium D-gluconate; yield 21.5 g. after drying at 80°. The crude product was recrystallized by solution in 100 cc. of boiling water, addition of 2 g. of active carbon, concentration of the filtrate to 50 cc. and addition of a small amount of ethanol. After sixteen hours in the icebox, the crystals were filtered, washed with 50% ethanol and dried; yield 20.2 g. Only 0.25 g. more was obtained from the mother liquor. The total yield of recrystallized product was 96% of theory. The rotation of the calcium D-gluconate in about 3% water solution was $[\alpha]^{20}_D + 8.5°$.

Nelson and Cretcher[101] applied the method of Hudson and Isbell to the oxidation of D-mannose and the crystalline lactone was prepared. A good yield was obtained in contrast to that reported in the earlier work of Fischer and Hirschberger[34] in which no control of *p*H was attempted. The method was also applied to the oxidation of starch by Felton, Farley and Hixon[102] and by Kihara.[102a]

The aldehydo acetates have been oxidized to the corresponding aldonic acids without deacetylation by the action of bromine in the presence of potassium bicarbonate. In this manner, Major and Cook[102b] obtained pentaacetyl-D-gluconic acid. A modification of this method was used by Wolfrom, Brown and Evans[102c] for the preparation of pentaacetyl-D-galactonic acid.

Iwadare[103] applied the method to *xylo*-trihydroxyglutaric aldehyde and its derivatives. The oxidation of the parent substance with bromine gave strontium *xylo*-trihydroxyglutarate, whereas a similar treatment of the D-1,2-isopropylidene derivative gave strontium 1,2-isopropylidene-D-xyluronate.

(101) W. L. Nelson and L. H. Cretcher, *J. Am. Chem. Soc.*, **52**, 403 (1930).

(102) G. Felton, F. F. Farley and R. M. Hixon, *Cereal Chem.*, **15**, 678 (1938).

(102*a*) Y. Kihara, *J. Agr. Chem. Soc. Japan*, **15**, 107 (1939).

(102*b*) R. T. Major and E. W. Cook, *J. Am. Chem. Soc.*, **58**, 2474 (1936).

(102*c*) M. L. Wolfrom, R. L. Brown, and E. F. Evans, *J. Am. Chem. Soc.*, **65**, 1021 (1943).

(103) K. Iwadare, *Bull. Chem. Soc. Japan*, **16**, 40 (1941).

The conversion of vicianose [6-(L-arabinosyl)-D-glucose] to the calcium vicianobionate was effected by oxidation with bromine in the presence of calcium carbonate. Bertrand and Weisweiller[104] then hydrolyzed the aldonic acid and obtained D-gluconic acid and L-arabinose, as evidence of the glycosidic nature of the L-arabinose unit in the disaccharide.

Attempts by Smith and Tollens[105] to oxidize D-fructose with bromine by the method of Clowes and Tollens[95] were unsuccessful; a double compound of D-fructose and calcium bromide was obtained. No yield of this product was given. The reaction mixture of D-fructose, calcium carbonate, water and bromine was shaken until all the bromine had "dissolved," after which the double compound was separated from the concentrated solution. The calcium bromide was formed from the interaction of calcium carbonate with hydrogen bromide in the reaction solution. The hydrogen bromide could be formed in two ways: by the hydrolysis of bromine in water (reaction 1, page 134) or by the reduction of bromine during the oxidation of D-fructose. The former is the more probable explanation, but a "blank run" without the ketose was not attempted.

4. *Electrolytic Oxidation*

In 1931, Isbell and Frush[106] reported the electrolytic oxidation of aldoses in the presence of calcium carbonate and a small amount of bromide, which served as a catalyst. The main solutes in the reaction mixture are the aldose and the calcium salt of the aldonic acid. The latter is easily crystallized from solution. The salts of D-gluconic, D-xylonic, lactobionic and maltobionic acids were prepared. A description of the process was published in 1932[107] and a patent[108] was issued in 1934. German and French patents[109] were issued in 1931 to the Rohm and Haas Co.[109]

The electrolytic method is an indirect oxidation, as the small amount of bromide present is constantly converted to bromine, the actual oxidant. Essentially the reaction is the same as the action of bromine in weakly acidic buffered solution. The presence of only a small amount of extraneous salts is of advantage in processing the reaction mixture. The

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(104) G. Bertrand and G. Weisweiller, *Compt. rend.*, **151**, 884 (1910).

(105) R. H. Smith and B. Tollens, *Ber.*, **33**, 1277 (1900).

(106) H. S. Isbell and Harriet L. Frush, *J. Research Natl. Bur. Standards*, **6**, 1145–52 (1931).

(107) H. S. Isbell, Harriet L. Frush and F. J. Bates, *J. Research Natl. Bur. Standards*, **8**, 571 (1932).

(108) H. S. Isbell, U. S. Pat. 1,976,731 (1934).

(109) Rohm and Haas Co., German Pat. 558,379 (1931); French Pat. 715,176 (1931); Edward L. Helwig, U. S. Pat. 1,895,414 (1933).

use of electrical energy is much more desirable in a commercial process than is the use of an excess of bromine.

The electrolytic preparation of D-gluconic acid is of considerable commercial interest because of the industrial importance of the calcium and other salts. The process can be carried out cheaply in a continuous manner by the slow addition of D-glucose and calcium carbonate to the cell and the removal of crystalline calcium D-gluconate. The product is of good purity. The various conditions for commercial production and discussion of the costs were investigated by Fink and Summers[110] and by Lal and Mukherji.[111] The latter[111] found that the development of too high an acidity led to the conversion of the D-gluconic acid to saccharic acid. Also the substitution of chloride ion for bromide ion caused much anode corrosion and a lower yield of D-gluconic acid.

Kiliani[112] reported very favorably on the electrolytic method and was quick to apply it to the preparation of calcium D-galactonate. In 1935, the electrolytic preparation of calcium D-xylonate was reported by Isbell and Frush[113]; the earlier work had been delayed by failure to obtain the salt in crystalline condition. The preparation of calcium lactobionate-calcium bromide was reported by Isbell[114]; the double salt was suggested as a sedative in place of calcium bromide.

"Over-oxidation" past the aldonic acid stage has been observed in several cases. As mentioned above, Lal and Mukherji[111] noted that the development of too high an acidity led to the production of saccharic acid. Pasternack and Regna[115] patented the preparation of calcium 2-keto-D-gluconate by the electrolytic oxidation of calcium D-gluconate in a weakly acid solution containing a soluble chromium compound and a chloride or bromide of an alkali or alkaline earth metal. No buffer was used. Niemann and Link[116] obtained a small yield (3%) of methyl α-D-galacturonide by the electrolytic oxidation of methyl α-D-galactopyranoside in buffered solution. Cook[117] patented the preparation of 2-keto aldonic acids by the electrolytic oxidation of osones in a buffered solution consisting of calcium carbonate and some calcium bromide.

(110) C. G. Fink and D. B. Summers, *Trans. Electrochem. Soc.*, **74**, preprint (1938); *C. A.*, **32**, 6955 (1938).

(111) J. B. Lal and K. C. Mukherji, *Dept. Ind. Com., United Provinces, Bull.* No. 29 (1941).

(112) H. Kiliani, *Ber.* **65**, 1269 (1932).

(113) H. S. Isbell and Harriet L. Frush, *J. Research Natl. Bur. Standards*, **14**, 359 (1935).

(114) H. S. Isbell, *J. Research Natl. Bur. Standards*, **17**, 331 (1936).

(115) R. Pasternack and P. P. Regna, U. S. Pat. 2,222,155 (1940).

(116) C. Niemann and K. P. Link, *J. Biol. Chem.*, **104**, 195 (1934).

(117) E. W. Cook, Canadian Pat. 398,202 (1941).

It was noticed by Cook and Major[118] that the reducing value of a D-glucose solution did not disappear entirely upon electrolytic oxidation, even when more current had been used than was theoretically necessary to form D-gluconic acid. This residual reducing action was due to 5-keto-D-gluconic acid, formed by the oxidation of D-gluconic acid. From 7 g. of D-glucose a yield of 0.8 g. of calcium 5-keto-D-gluconate was obtained.

It should be noted that twenty-two years before the method of Isbell and Frush was reported, Löb[119] had oxidized D-glucose electrolytically in 0.5 N acid solution, but considerable degradation was observed. In this work no bromide ion was present. The calcium content of the precipitated salt was always greater than that of calcium D-gluconate (9.32%) and approached that of calcium trihydroxyglutarate (18.4%). Formaldehyde and formic acid were also isolated. Neuberg[120] observed a strong degradation of various sugar acids; he had hoped to obtain a Kolbe electrosynthesis of C_{10} alcohols from D-gluconic acid:

$$2R{-}COO^- \rightarrow R{-}R + 2CO_2 + 2e \qquad (13)$$

but instead D-arabinose was obtained, and similar degradations were observed with the other aldonic acids.

Preparation of Calcium D-Gluconate by Electrolytic Oxidation.[106] A solution of 45 g. (0.25 mole) of anhydrous D-glucose and 8 g. of calcium bromide in 1 liter of water is stirred in a three-necked flask with 12.5 g. of calcium carbonate. The flask is equipped with two graphite electrodes, 22 mm. in diameter and sufficiently long to reach the bottom of the flask and connected with a direct current source of 5 to 10 volts. A current of 0.5 ampere is passed through the solution and the quantity of electricity measured. The extent of oxidation is nearly proportional to the quantity of electricity used and the reaction is virtually complete when the theoretical amount (13.4 ampere-hours) has passed through the reaction mixture. When a very weak reducing value is obtained, the solution is filtered, and concentrated to a thin sirup from which crystalline calcium D-gluconate separates readily. The yield is about 45 g., and the product can be recrystallized from hot water. The mother liquor may be returned to the electrolytic cell and used for further runs after the addition of more D-glucose and calcium carbonate.

5. *Oxidation in Alkaline Solution*

The action of the hypohalites is generally far more drastic in alkaline solution than in acid or neutral media. As the values of the oxidation potentials (Table I) do not predict this result, the effect may be attributed in part to the action of alkali on the sugars. The oxidation is not con-

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(118) E. W. Cook and R. T. Major, *J. Am. Chem. Soc.*, **57**, 773 (1935).

(119) W. Löb, *Biochem. Z.*, **17**, 132 (1909).

(120) C. Neuberg, *Biochem. Z.*, **7**, 537 (1908); C. Neuberg, L. Scott and S. Lachman, *ibid.*, **24**, 152 (1910).

fined to the reducing group alone. If rigid controls are not maintained, the primary and secondary alcohol groups are also attacked with the resulting formation of keto acids and cleavage of carbon-carbon bonds. Ketoses can also be broken into fragments. The action is complicated by the effect of the alkali on the oxidant as well as on the sugar; the oxidants tend to decompose into various products including the inactive halogenates.

a. Alkaline Hypoiodite as an Analytical Reagent. The use of this oxidant was initiated by Romijn,[121] who discovered that it would oxidize aldoses "stoichiometrically" to aldonic acids (reaction 14). If the conditions were carefully controlled, quantitative results were obtained. The stoichiometrical relationship was a great advantage in comparison to other reagents such as the alkaline copper solution, wherein "over-oxidation" required the application of empirical factors. As the reaction is complicated by the tendency of the hypoiodite to be converted into iodate in alkaline solution, the reaction was buffered with borax and

$$RCHO + I_2 + 3NaOH \rightarrow RCOONa + 2NaI + 2H_2O \qquad (14)$$

allowed to run at 25° for eighteen hours. The lower *p*H gave a more stable solution (see Table IV, page 139) but also reduced the speed of the oxidation.

As this lengthy reaction time proved objectionable from the analytical viewpoint, certain modifications were applied. Willstätter and Schudel[122] shortened the time to twenty minutes by adding a definite excess of iodine (100%) and of alkali (150%); the latter was added slowly to a mixture of the sugar and iodine. In this way the formation of iodate was lessened and "over-oxidation" minimized.

Procedure of Willstätter and Schudel.[122,122a] To 10 cc. of a solution containing 100 mg. of aldose is added 20 cc. of 0.1 *N* iodine-potassium iodide solution (11.11 cc. is required for a hexose) and then 30 cc. of 0.1 *N* sodium hydroxide solution is added gradually. The mixture is allowed to stand for ten to twenty minutes. It is then acidified with dilute hydrochloric or sulfuric acid and titrated with 0.1 *N* thiosulfate solution. Each cc. of 0.1 *N* iodine consumed is equivalent to 9.005 mg. of an aldohexose, 7.004 mg. of an aldopentose and 17.11 mg. of a reducing C_{12} disaccharide.

Goebel[123] made a study of the effect of the rate of addition of alkali upon the formation of iodate from hypoiodite (reaction 15). He found

$$3NaOI \rightarrow NaIO_3 + 2NaI \qquad (15)$$

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(121) G. Romijn, *Z. anal. Chem.*, **36**, 349 (1897).

(122) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918).

(122*a*) C. A. Browne and F. W. Zerban, "Physical and Chemical Methods of Sugar Analysis," John Wiley and Sons, Inc., New York, p. 896 (1941).

(123) W. F. Goebel, *J. Biol. Chem.*, **72**, 801 (1927).

that the rate of iodate formation was independent of the rate of alkali addition in the absence of aldose, but that there was a very definite effect when the sugar was present. Also, for reaction 14 to go to completion, a sufficient concentration of alkali must be present. Thus, when alkali is added immediately to a D-glucose-iodine mixture, a high hypoiodite concentration is not realized because reaction 15 enters the picture with the formation of inactive iodate. If the alkali is added over a period of two minutes, the greater part of the hypoiodite will react with the sugar rather than form iodate, for the initial speed of reaction 14 is greater than that of reaction 15. Since the over-all speed of reaction 15 is greater than that of reaction 14, however, the iodate formation will eventually win out over the oxidation of the aldose, unless the alkali is added gradually.

Kline and Acree[124] studied the hypoiodite oxidation extensively. The alkali and iodine were both added in small portions throughout the reaction. In this manner the concentration of sugar relative to the sodium hypoiodite was kept at a level favorable to the sugar oxidation (reaction 14). At the end of the reaction, the formation of iodate increased rapidly (reaction 15), taking precedence over the oxidation of ketoses and non-reducing sugars.

The earlier work on this oxidation by Slater and Acree[124a] was in error because of their failure to distinguish between the iodine equivalence of thiosulfate in acid and in alkaline solution. In acid solution (reaction 15*a*) the equivalence is 1:1, with sodium tetrathionate as a product. In alkaline solution 1 mole of thiosulfate is equivalent to 8 atoms of iodine (reaction 15*b*), with sodium sulfate as a product.

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6 \qquad (15a)$$

$$4I_2 + Na_2S_2O_3 + 10NaOH \rightarrow 2Na_2SO_4 + 8NaI + 5H_2O \qquad (15b)$$

Procedure of Kline and Acree.[124] For the determination of the aldose present it is preferable to take an aliquot of the sugar solution, or a weighed amount of the solid substance, which will react with approximately 20 ml. of 0.1 *N* iodine. Titrate this solution with 0.1 *N* sodium hydroxide or hydrochloric acid until it is exactly neutral to phenolphthalein. Add the phenolphthalein at this point only when it is necessary to bring the solution to neutrality and use only one drop, as the alcohol introduces a potential source of error involving a loss of iodine. A water solution of this indicator or of phenol red or thymol blue might be used for this titration. Add 5 ml. of 0.1 *N* iodine from a buret; then add drop by drop from a buret 7.5 ml. of 0.1 *N* sodium hydroxide. Repeat this process until 22 ml. of iodine and 35 ml. of alkali have been added. This operation takes about five to six minutes. Allow a two-minute interval for the completion of the oxidation. Acidify with 0.1 *N* (or 0.2 *N*) hydrochloric acid to free the iodine from any sodium iodate present and titrate the liberated iodine with

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(124) G. M. Kline and S. F. Acree, *J. Research Natl. Bur. Standards*, **5**, 1063 (1930).

(124*a*) C. S. Slater and S. F. Acree, *Ind. Eng. Chem., Anal. Ed.*, **2**, 274 (1930).

0.1 *N* thiosulfate; a starch indicator may be used. Add two to three drops of phenolphthalein solution and titrate the excess acid with 0.1 *N* sodium hydroxide.

If the iodine liberated by acidification requires more than 2–3 ml. of 0.1 *N* thiosulfate, too much iodine has been added, resulting in "over-oxidation." If a very accurate determination is desired, the experiment is repeated and the amount of the thiosulfate titration less 2 ml. is deducted from the amount of iodine to be added to the unknown. On the other hand, if the thiosulfate titration after acidification is less than 1.5 ml., a sufficient amount of iodine has not been added. In such a case it is necessary to repeat the experiment adding more iodine and alkali.

The number of ml. of 0.1 *N* iodine minus the number of ml. of 0.1 *N* thiosulfate, and the number of ml. of 0.1 *N* sodium hydroxide minus the number of ml. of 0.1 *N* hydrochloric acid give the respective quantities of 0.1 *N* iodine and 0.1 *N* sodium hydroxide used in the oxidation of the sugar. One millimole of the sugar (0.15 g. of D-xylose) requires for oxidation 20 ml. of 0.1 *N* iodine and 30 ml. of 0.1 *N* sodium hydroxide (reaction 14, page 157). Either iodine or alkali consumed, or both as checks, may be used to calculate the amount of aldose present.

The ketose and non-reducing sugars are not oxidized sufficiently to interfere with the precision of the method unless iodine and alkali are used in excess of the above amounts. Lignins and other compounds oxidized by iodine and alkali generally react differently than sugars and disturb the 2:3 ratio for iodine and alkali.

Considerable work has been done on the velocity of oxidation of aldoses by sodium hypoiodite. Myrbäck[125] noted that maltose was oxidized about one-fifth slower than D-glucose in sodium hydroxide solution, and two-fifths slower when a buffer (sodium carbonate-sodium bicarbonate) was used. When borate buffers were used,[126] the rate of oxidation for D-glucose was slowed down greatly, being only 5% of that without a borate buffer at the same *p*H. It was assumed that compound formation with the borate took place, an addition complex involving the first and second carbon atoms being postulated. This was based on the fact that the rate of oxidation of methylated sugars (2,3,6-trimethyl-D-glucose and 2,3,4,6-tetramethyl-D-glucose) with the second carbon atom blocked was the same in borate and other buffers. The rate for the methylated sugars was about two-thirds of that observed for D-glucose.

The restriction of the oxidizing action of sodium hypoiodite to the —CHO group and the low amount of actual "over-oxidation" was pointed out by Sobotka.[127] The equivalents of oxygen consumed by D-glucose and by various methylated derivatives when oxidized by sodium hypoiodite and by two alkaline copper reagents were determined (Table V). The maximum variation for sodium hypoiodite was 9% (2,3,4,6-tetramethyl-D-glucose), a value which was very small compared with that shown by the other oxidants. With the copper reagents the greatest deviation was shown when the second carbon atom carried a

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(125) K. Myrbäck, *Svensk Kem. Tid.*, **51,** 149 (1939).

(126) K. Myrbäck and E. Gyllensvard, *Svensk Kem. Tid.*, **54,** 17 (1942).

(127) H. Sobotka, *J. Biol. Chem.*, **69,** 267 (1926).

methoxyl group. Apparently "over-oxidation," so prevalent with the copper reagents, is blocked by a methoxyl group on the second carbon atom.

TABLE V

Reducing Power of Substituted Sugars[127]

Sugar	*Method of Analysis*		
	Willstätter-Schudel	*Lehmann-Maquenne*	*Bertrand*
D-Glucose	1.99	5.16	5.41
3-Methyl-D-glucose	1.98	3.21	3.66
2,3-Dimethyl-D-glucose	2.00	0.59	1.94
3,5,6-Trimethyl-D-glucose	1.96	1.98	2.07
2,3,4,6-Tetramethyl-D-glucose	1.81	0.82	1.22
2,3,4,5,6-Pentamethyl-D-glucose	2.02	1.49	1.60

The procedures, like the Willstätter-Schudel and its modifications by Goebel and Acree, that involve the use of strong alkali, are difficult to carry out in a reproducible manner. Cajori[127a] has recommended the employment of an iodine-sodium carbonate system which is very useful. A recent procedure employing borate buffer is that reported by Harris and coworkers[127b] and applied especially to oxycelluloses.

The analytical work using alkaline hypoiodite may be summarized as follows. Because of "over-oxidation" and the formation of iodate, the reaction is not always quantitative, being stoichiometrical only when conditions are carefully controlled. It is subject to additional errors when alcohols and sugars other than aldoses are present. It can be employed in analytical work but is subject to limitations and should be used with caution.

b. Effect of Sodium Hypoiodite on Ketoses. When Romijn[121] reported his first work on the oxidation of the aldoses, he noted that sodium hypoiodite did not oxidize ketoses to any great extent. This difference in rate was utilized to differentiate between the two classes of sugars. Kolthoff[128] found that D-fructose absorbed 1.2 cc. of 0.1 N iodine solution per gram of sugar and sucrose 0.35 to 0.6 cc. depending on the time of reaction and the concentration. Auerbach and Bodländer[129] used a buffered solution of sodium carbonate and bicarbonate at pH 10.1–10.2

(127*a*) F. A. Cajori, *J. Biol. Chem.*, **54**, 617 (1922).

(127*b*) H. A. Rutherford, F. W. Minor, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **29**, 131 (1942).

(128) I. M. Kolthoff, *Z. Untersuch. Nahr. u. Genussm.*, **45**, 131 (1923).

(129) F. Auerbach and E. Bodländer, *Z. angew. Chem.*, **36**, 602 (1923); *Z. Untersuch. Nahr. u. Genussm.*, **47**, 233 (1925).

to decrease the oxidation of ketoses; the error for 100 mg. of D-fructose was equivalent to 1 mg. of D-glucose, and the error for sucrose was negligible. However, other investigators have been unable to reproduce this work; the oxidation was not complete in the specified time, and a longer time was objectionable.

A definite method for the determination of D-fructose in the presence of aldoses was worked out by Kruisheer[130]; 98.3% of theory was found. The aldoses were oxidized with sodium hypoiodite, after which the excess iodine was titrated with sodium sulfite. Thiosulfate could not be used here, since the subsequent determination of D-fructose was carried out with an alkaline copper solution by the Luff-Schoorl[131] method.

The above methods are so designed that the oxidation of ketoses by sodium hypoiodite is restricted to a minimum. Bailey and Hopkins[132] studied the conditions under which ketoses would be oxidized. The reaction rate increased with the temperature, and the extent of the reaction, which was studied over the temperature range of 1 to 35°, went through a minimum at 15°. An excess of alkali in the 17–37° range apparently caused enolization of the D-fructose and increased the extent of the oxidation. When the alkali was added progressively in small amounts, this effect was increased 4–5 times and was independent of the D-fructose concentration. Oxalic acid was isolated and the presence of D-erythronic acid was assumed.

c. Preparation of Aldonic Acids. Oxidation in alkaline solution has been utilized to some extent for the preparation of the aldonic acids as well as for analytical work. Goebel[133] prepared calcium D-gluconate and maltobionate by treatment of the corresponding aldoses with barium hypoiodite. Levene and Wolfrom[134] used this method to prepare cellobionic acids. Good yields were obtained. Ochi[135] obtained a 50% yield of D-gluconic acid by the action of bleaching powder; the low yield was due to degradation products, such as oxalic and saccharic acids. Moore and Link[136] used a methanol solution of potassium hypoiodite to prepare the potassium salts of aldonic acids as intermediates for the characterization of the acids as benzimidazole derivatives. A 92% yield of the D-gluconate and good yields of the L-arabonate and D-galactonate were obtained. D-Mannose, D-xylose, L-rhamnose and D-lyxose were oxidized

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(130) C. I. Kruisheer, *Z. Untersuch. Nahr. u. Genussm.*, **58**, 261 (1929).

(131) N. Schoorl, *Chem. Weekblad.*, **22**, 132 (1925).

(132) K. Bailey and R. H. Hopkins, *Biochem. J.*, **27**, 1965 (1933).

(133) W. F. Goebel, *J. Biol. Chem.*, **72**, 809 (1927).

(134) P. A. Levene and M. L. Wolfrom, *J. Biol. Chem.*, **77**, 671 (1928).

(135) S. Ochi, *Proc. World Eng. Congr., Tokyo*, 1929, **31**, 537 (1931).

(136) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 293 (1940).

similarly, but the barium salts were isolated, since the potassium salts were soluble in methanol. The potassium salts could be recrystallized from water but were generally used as intermediates and converted directly to the desired derivatives.

Hönig and Ruzicka[137] oxidized both D-glucose and D-galactose to the aldonic acids with barium hypobromite in alkaline solution. Usually additional alkali was added to the solution during the course of the reaction as the products were acidic. Ultraviolet light was used as a catalyst, and the reaction was complete in forty-five minutes. The lower the concentration of sugar and alkali (0.1 *N* alkali and 0.1% sugar), the more uniform was the reaction. The acids were isolated in 83 and 80% yields as the calcium salts. An excess of calcium carbonate was avoided in order to prevent darkening. This behavior is reminiscent of the instability of keto acids. The oxidation was also carried out with 0.1 *N* calcium hypochlorite, but the reaction was slower (three hours for a negative test with starch-iodide paper to be obtained) and the yields were poorer, being 61 and 58%. The oxidation with hypochlorite was simpler in its technique, however, as the calcium salts were obtained directly.

The production of salts of aldonic acids by the action of chlorine in the presence of small amounts of bromide or iodide was patented by the Chemische Fabrik vorm. Sandoz.[138] The solution was kept alkaline with sodium carbonate, alkali being added during the reaction to prevent the loss of bromine vapor. This reaction apparently involves an oxidation by bromine, the chlorine serving as an indirect oxidant, as in the method of Ling and Nanji.[98] In the latter method a slow stream of chlorine is passed into a buffered weakly acidic solution of bromide and calcium carbonate.

d. "Over-oxidation" of Aldoses. The prolonged oxidation of an aldose converts the primary product, an aldonic acid, to a keto aldonic acid, which subsequently splits off carbon dioxide and forms a lower aldonic acid. Hönig and Tempus[139] claimed to have oxidized D-glucose in steps with barium hypobromite to D-gluconic, 2-keto-D-gluconic and D-arabonic acids. The results are shown in Table VI; too large an excess of alkali and too high alkali concentrations were unfavorable for the formation of these three acids. A high sugar concentration also led to low yields. To minimize the effect of the alkali, the reactions were "accelerated" with sunlight. These findings were disputed by Reichstein and Neracher,[140] who claimed that 5-keto-D-gluconic acid was formed, and not the

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(137) M. Hönig and W. Ruzicka, *Ber.*, **62**, 1434 (1929).

(138) Chemische Fabrik vorm. Sandoz, French Pats. 635,603 (1928), 656,863 (1929).

(139) N. Hönig and F. Tempus, *Ber.*, **57**, 787 (1924).

(140) T. Reichstein and O. Neracher, *Helv. Chim. Acta*, **18**, 892 (1935).

2-keto acid; traces of the latter were found but the primary product was shown to possess a 5-keto group by oxidation to *xylo*-trihydroxyglutaric acid. Ruzicka[141] also disagreed with Hönig and Tempus; the products always possessed lower reducing power than reported originally and the phenylhydrazone of the keto acid hydrazide could not be prepared. He concluded that the oxidation products were not uniform. Also, Hönig and Ruzicka worked together[137] and noted that the formation of the aldonic acids by oxidation with hypobromite was favored by low alkali and sugar concentrations.

TABLE VI

Stepwise Oxidation of D-*Glucose with Barium Hypobromite*[139]

Concentration			*Equivalents per mole of* D-*glucose*		*Time, min.*	*Yield of main product, %*
Ba(OBr)$_2$, N	D-*Glucose, %*	*Ba(OH)$_2$, N*	*Ba(OH)$_2$,*	*Oxygen consumed*		
0.1	1	0.1	1.1	1	45	78 (D-gluconic acid)
0.5	1	0.1	1.5	1	25	65 "
0.1	1	0.1	2.0	1	20	63 "
0.1	10	0.1	1.1	1	45	69 "
0.02	0.1	0.02	1.0	1	20	87 "
0.1	1	0.1	1.1	1	—	84 "
0.1	1	0.1	1.3	2	90	61 (2-keto-D-gluconic acid)
0.5	1	0.1	1.9	2	—	49 "
0.1	10	0.1	1.0	2	—	49 "
0.02	0.1	0.02	1.3	2	—	68 "
0.1	1	0.1	1.3	2	—	63 "
0.1	1	0.1	4.0	3	570	42 (D-arabonic acid)
0.5	1	0.1	6.0	3	—	33 "
0.1	1	0.1	8.0	3	450	17 "
0.1	10	0.1	4.5	3	570	35 "
0.02	0.1	0.02	4.5	3	540	53 "
0.1	1	0.1	4.0	3	—	47 "
0.1	1	0.1	2.7	2	90	20 (2-keto-D-gluconic acid) 22 (D-arabonic acid)

The preparation of 2-keto-D-gluconic acid from D-glucosone was reported by Myrbäck.[142] The osone was oxidized in alkaline solution

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(141) W. Ruzicka, *Z. Zuckerind. Čecheslovak. Rep.*, **64**, 219 (1941).

(142) K. Myrbäck, *Svensk Kem. Tid.*, **51**, 225 (1939).

with hypoiodite under conditions similar to that for D-glucose; as the oxidation velocity was the same, the 2-keto group appears to have no particular effect on the aldehyde group. The osone was less stable in sodium hydroxide solution than was D-glucose, but in an alkaline solution of sodium hydroxide and sodium bicarbonate this effect apparently was minimized, for the oxidation rates were about the same.

Bergmann and Wolff[143] reported a small yield (1 g.) of menthol α-D-glucuronide by the oxidation of 12 g. of menthol α-D-glucoside in pyridine solution with sodium hypobromite. Better yields were obtained by Smolenski,[144] who oxidized methyl α-D-glucopyranoside with bromine and sodium carbonate; a 30% yield of the methyl D-glucuronide (as the brucine salt) was reported. Jackson and Hudson[145] obtained a 12% yield of the brucine salt of methyl α-D-mannuronide by the barium hypobromite oxidation of methyl α-D-mannopyranoside.

The last reaction was non-selective; it was carried out for sixteen to twenty days at a low temperature (3–7°), and 25–30% of the unaltered starting material was recovered. A 25% yield of strontium D-methoxy-D′-hydroxymethyldiglycolate dihydrate showed that the reagent attacked C2, C3 and C4, and split out formic acid at C3. This reaction is similar to the familiar one observed with periodic acid[1] except that the product is a diacid and not a dialdehyde.

A further degradation of methyl α-D-glucoside by the action of barium hypobromite was observed by Bergmann and Wolff.[143] From 12 g. of the glycoside, 4.6 g. of glyoxylic acid benzylphenylhydrazone was obtained. The experimental conditions differed from those used in the preparation of menthol α-D-glucuronide in that about twice as much bromine was used.

The primary hydroxyl group in 2,3:4,6-diisopropylidene-L-sorbose was oxidized with sodium hypochlorite in the presence of $NiCl_3$ by Weijlard,[146] who obtained a 90% yield of 2,3:4,6-diisopropylidene-2-keto-L-gulonic acid monohydrate. This reaction is normally carried out with permanganate for the production of ascorbic acid, but a war-time shortage of this oxidant caused the consideration of chlorine as a substitute. Similar results were found by Beer and Preobrazhenskii[146a] in more concentrated solutions. The yield of product was lowered drastically when the concentration of sodium hydroxide was less than 30%, when the chlorine-sorbose molar ratio was less than 4.1 and when

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(143) M. Bergmann and W. W. Wolff, *Ber.*, **56**, 1060 (1923).

(144) K. Smolenski, *Roczniki Chem.*, **3**, 153 (1924).

(145) E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 994 (1937).

(146) J. Weijlard, *J. Am. Chem. Soc.*, **67**, 1031 (1945).

(146*a*) A. A. Beer and N. A. Preobrazhenskii, *J. Appl. Chem.* (*U. S. S. R.*), **19**, 1121 (1946).

the temperature was allowed to rise above 60°. If the amount of catalyst (both nickel and cobalt chlorides) was above 4% of the weight of starting material, the reaction became too violent.

The oxidative degradation of amides of the sugar series with bromine was investigated by Bergmann.[147] The monamide of mucic acid was treated with potassium hypobromite and a lyxuronic acid was obtained as the hydrazide. This is the well-known Hofmann reaction (equations 16 and 17). The Weerman reaction[148] (equations 18 and 19), for the detection of α-hydroxy amides has been applied by Ault, Haworth and Hirst[149] to a determination of the structure of the methylated sugar amides. Methylated derivatives substituted at C2 do not give sodium isocyanate, but when C2 is not blocked, sodium isocyanate is formed. Thus, 2,4-dimethyl-D-erythronamide and 2,3,5-trimethyl-L-arabonamide give no sodium isocyanate on treatment with sodium hypobromite, whereas 3,4-dimethyl-L-threonamide, with a free α-hydroxyl group, does.

$$RCONH_2 + NaOCl \rightarrow RNCO + NaCl + H_2O, \qquad (16)$$

$$RNCO + H_2O \rightarrow (RNHCOOH) \rightarrow RNH_2 + CO_2; \qquad (17)$$

$$RCHOHCONH_2 + NaOCl \rightarrow RCHOH{-}NCO + NaCl \qquad (18)$$

$$RCHOH{-}NCO + NaOH \rightarrow RCHO + NaNCO + H_2O \qquad (19)$$

It is interesting to note the products obtained when the α-hydroxyl group is blocked and the hydroxyl at C3 or C4 is free; a reaction between the isocyanate group and the free hydroxyl group in the same molecule results in the formation of a cyclic urethane.

Ochi[135] showed that calcium hypochlorite (0.05 *M*) will oxidize D-fructose; not more than 37% of the starting material was consumed, even at 45°. A solution of D-glucose and D-fructose was oxidized, and 80% of the D-glucose and 11% of the D-fructose were attacked. Similarly 92.4% of D-glucose and 19% of sucrose in a mixture of the two sugars were oxidized.

Bleyer and Braun[150] oxidized D-glucose with chloramine in alkali; under these conditions the chloramine was converted to the amide and sodium hypochlorite, the action of the latter being very drastic. Each mole of aldose consumed 8 equivalents of oxygen, liberated 2 moles of carbon dioxide and formed 2 equivalents of an unknown acid. The reaction went more rapidly as the alkalinity was increased. The acid was assumed to be acetic acid, formed from pyruvic acid; D-gluconic acid was considered to be the primary oxidation product. Bernhauer and

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(147) M. Bergmann, *Ber.*, **54**, 1376 (1921).

(148) R. A. Weerman, *Rec. trav. chim.*, **37**, 16 (1917).

(149) R. G. Ault, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1722 (1934).

(150) B. Bleyer and W. Braun, *Biochem. Z.*, **180**, 105 (1927); **183**, 310 (1927); **199**, 186 (1928).

Schön[151] found no trace of acetic acid but isolated the lead salt of formic acid. They also found that D-gluconic acid was not attacked by chloramine under the conditions described by Bleyer and Braun.

e. Oxidation of Sugar Alcohols. Glycerol has been converted to "glycerose" by the action of bromine and sodium carbonate; Fischer and Tafel[152] obtained a yield of more than 20%, based on the glycerose phenylosazone formed from the oxidation products. The product was considered to be a mixture of D,L-glyceraldehyde and dihydroxyacetone. A later method[153] gave much better yields; glycerol was treated with lead hydroxide to form lead glycerate, which was treated, in solid form, with bromine vapor. The absence of D,L-erythronic acid in the reaction products of "glycerose" with hydrogen cyanide, indicated that the amount of D,L-glyceraldehyde in "glycerose" was small. Wohl and Neuberg[154] determined the amount of D,L-glyceraldehyde in the oxidation products by means of a glyceraldehyde-phloroglucinol derivative. Small amounts of the aldehyde were found in the bromine-oxidized lead glycerate product but none was found in the product resulting from the action of bromine and sodium carbonate on glycerol. The effect of alkali on any possible D,L-glyceraldehyde produced by the bromine-sodium carbonate oxidation was shown to be negligible in the short time of the oxidation.

No reaction occurs between glycerol and sodium hypochlorite unless a trace of cobalt acetate[155] is added; with this catalyst present, formaldehyde is formed at low temperatures. With neutral hypochlorite, even in the presence of cobalt acetate, no reaction occurs. The same formation of formaldehyde is observed with calcium hypochlorite and cobalt acetate.

Dulcitol was oxidized by Fischer and Tafel[152] with bromine and sodium carbonate; the product reacted with phenylhydrazine to form a phenylosazone similar to D-galactose phenylosazone but with a higher melting point (205–6° instead of 196°). Similarly, Fischer[156] converted xylitol to a product that gave crystalline D,L-xylose phenylosazone. Volemitol[157] (α-sedohepitol) gave a product that formed a 20% yield of a heptose phenylosazone. Neuberg[35] reported the preparation of an optically inactive tetrose from erythritol; the yield was better than that obtained by oxidation with nitric acid and was determined by measuring the amount of erythrose phenylosazone subsequently formed.

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(151) K. Bernhauer and K. Schön, *Biochem. Z.*, **202**, 159 (1928).

(152) E. Fischer and J. Tafel, *Ber.*, **20**, 3384 (1887).

(153) E. Fischer and J. Tafel, *Ber.*, **22**, 106 (1889).

(154) A. Wohl and C. Neuberg, *Ber.*, **33**, 3095 (1900).

(155) H. P. den Otter, *Rec. trav. chim.*, **56**, 474 (1937).

(156) E. Fischer, *Ber.*, **27**, 2486 (1894).

(157) E. Fischer, *Ber.*, **28**, 1973 (1895).

In the case of the oxidation of glycerol, D,L-glyceric acid has also been isolated. Hlasiwetz and Habermann[24] treated glycerol with chlorine water and obtained the acid as the main product. From the action of bromine water on glycerol, Barth[158] obtained glyceric acid, bromoform and carbon dioxide. In the absence of water, bromine converted glycerol to dibromohydrin and bromoacetic acid. The former decomposed to hydrogen bromide and acrolein.

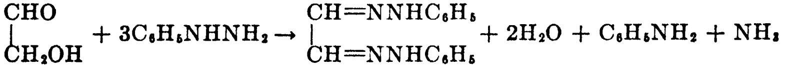
6. *Miscellaneous Action of Halogens on Simple Carbohydrates*

The possibility of forming halogen derivatives of the carbohydrates by substitution reactions was considered from the first work of Hlasiwetz and Habermann. Many workers postulated a brominated sugar as an intermediate in the formation of aldonic acids; the subsequent neutralization of the solution with silver oxide supposedly removed the halogen. However, all later work showed that no substitution took place in aqueous systems. On the other hand, Fenton and Gostling[159] found that a reaction occurred when D-fructose was treated with ether saturated with dry hydrogen bromide; heat often developed and an intense purple color was formed. The latter was due to the formation of 5-(bromomethyl)-2-furaldehyde which gives this color with ether solutions of hydrogen bromide. The same color was observed with other ketoses and with D-xylose. Aldoses gave duller colors and polyhydric alcohols yielded only a yellow color.

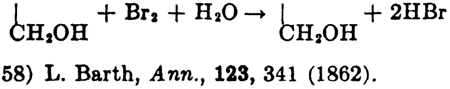
The non-aqueous oxidation of various ketones and aldehydes to hydroxyaldehydes and hydroxyketones has been carried out in two steps; the first step is a bromination and the second a hydrolysis, or replacement of the bromine atoms by hydroxyl groups. Fischer and Landsteiner[160] brominated acetal in the presence of calcium carbonate (reaction 20). The bromoaldehyde was then treated with cold barium hydroxide solution (reaction 21); the resulting glycolic aldehyde was identified by conversion to glyoxal phenylosazone (reaction 22) and by the formation of calcium glycolate, after oxidation with bromine (reaction 23).

$$CH_3CH(OC_2H_5)_2 + Br_2 \rightarrow BrCH_2CH(OC_2H_5)_2 + HBr \qquad (20)$$

$$BrCH_2CH(OC_2H_5)_2 + 3H_2O \rightarrow HOCH_2CHO + HBr + 2C_2H_5OH \qquad (21)$$



$$\begin{array}{l} CHO \\ | \\ CH_2OH \end{array} + 3C_6H_5NHNH_2 \rightarrow \begin{array}{l} CH{=}NNHC_6H_5 \\ | \\ CH{=}NNHC_6H_5 \end{array} + 2H_2O + C_6H_5NH_2 + NH_3 \qquad (22)$$



$$\begin{array}{l} CHO \\ | \\ CH_2OH \end{array} + Br_2 + H_2O \rightarrow \begin{array}{l} COOH \\ | \\ CH_2OH \end{array} + 2HBr \qquad (23)$$

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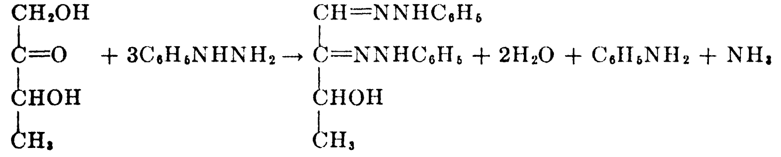
(158) L. Barth, *Ann.*, **123**, 341 (1862).

(159) H. J. H. Fenton and Mildred Gostling, *J. Chem. Soc.*, **75**, 423 (1899).

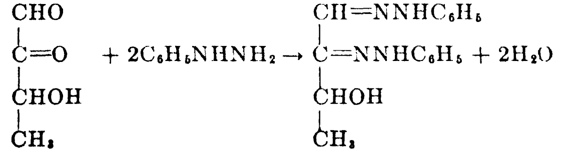
(160) E. Fischer and K. Landsteiner, *Ber.*, **25**, 2549 (1892).

Acetone was converted to monobromoacetone by Nef[161]; hydrolysis with potassium formate in methanol gave monohydroxyacetone very smoothly. The substituted hydroxyacetone, $CH_3COCHOHCH_3$, could not be brominated directly. However, the corresponding benzoate was brominated by Diels and Stephan[162] to the mono and dibromo derivatives, $CH_2BrCOCH(OBz)CH_3$ and $CHBr_2COCH(OBz)CH_3$. The two compounds were hydrolyzed at 0° with dilute alkali and a desoxyketotetrose and a desoxytetrosone formed. The former was isolated as a strongly-reducing, yellow sirup and was converted to the phenylosazone (reaction 24). The latter was obtained in solution only and was also converted to the same phenylosazone (reaction 25). The phenylosazone was similar to that obtained from 3-C-methylglyceraldehyde by Wohl and Frank[163] (reaction 26).



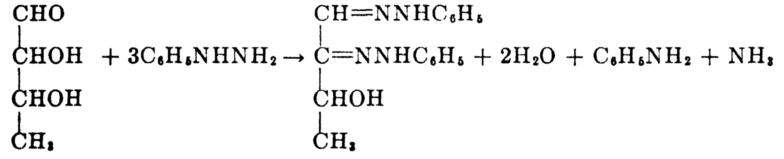
$$\begin{array}{l} \mathrm{CH_2OH} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{3C_6H_5NHNH_2} \rightarrow \begin{array}{l} \mathrm{CH{=}NNHC_6H_5} \\ | \\ \mathrm{C{=}NNHC_6H_5} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{2H_2O} + \mathrm{C_6H_5NH_2} + \mathrm{NH_3} \qquad (24)$$

Desoxyketotetrose



$$\begin{array}{l} \mathrm{CHO} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{2C_6H_5NHNH_2} \rightarrow \begin{array}{l} \mathrm{CH{=}NNHC_6H_5} \\ | \\ \mathrm{C{=}NNHC_6H_5} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{2H_2O} \qquad (25)$$

Desoxytetrosone



$$\begin{array}{l} \mathrm{CHO} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{3C_6H_5NHNH_2} \rightarrow \begin{array}{l} \mathrm{CH{=}NNHC_6H_5} \\ | \\ \mathrm{C{=}NNHC_6H_5} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{2H_2O} + \mathrm{C_6H_5NH_2} + \mathrm{NH_3} \qquad (26)$$

Desoxytetrose
(3-C-Methylglyceraldehyde)

Addition of bromine to a double bond has been observed in several cases. The hydrolysis of the resulting dibromide leads to a substance which may be regarded as an oxidation product of the original unsaturated compound. Fischer and Dangschat[164] prepared glycolic aldehyde triacetate by the action of bromine on vinyl acetate and treatment of the resulting dibromide with acetic acid.

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(161) J. U. Nef, *Ann.*, **335**, 247 (1904).
(162) O. Diels and E. Stephan, *Ber.*, **42**, 1787 (1909).
(163) A. Wohl and F. Frank, *Ber.*, **35**, 1907 (1902).
(164) H. O. L. Fischer and Gerda Dangschat, *Ber.*, **62**, 862 (1929).

Another addition reaction was observed in the case of 3,5-diacetyl-L-arabinal. A chloroform solution of this substance was treated with bromine by Gehrke and Aichner,[165] who isolated crystals of the unstable 1,2-dibromide. On the other hand, when sirupy L-arabinal was oxidized with bromine in aqueous solution, the resulting product was L-ribonic acid, isolated as the cadmium salt and as the lactone. Fischer, Bergmann and Schotte[166] prepared the 1,2-dibromide and the 1,2-dichloride of D-glucal triacetate in a similar manner. The dibromide was very unstable; a crystalline product was prepared only once and it quickly reverted to an oil. The crystalline dichloride was more stable and was slightly soluble in hot water. The aqueous solution was acidic and on cooling an oil separated. The halogen on C1 was easily removed from either the dibromide or the dichloride, a behavior contrasting with that of the halogen on C2. Silver acetate in acetic acid replaced the halogen at C1 with an acetate group; for example, the 1,2-dibromide of D-glucal triacetate gave tetraacetyl-2-desoxy-2-bromo-D-"glucose." Again, with methanol and silver carbonate two isomeric methyl triacetyl-2-desoxy-2-bromo-D-glucosides were formed. The bromine on C2 was not removed by aqueous silver nitrate at 100°; alkali removed the halogen, and treatment with 25% ammonium hydroxide at 100° replaced it with the amino group. Extended hydrolysis of methyl 2-desoxy-2-bromo-D-glucoside with N hydrochloric acid at 100°, followed by treatment with phenylhydrazine, gave D-glucose phenylosazone. Hence, the conversion of D-glucal to D-glucose phenylosazone might be considered as an oxidation to the D-glucose or D-mannose stage. This has been accomplished directly by the oxidation of D-glucal with perbenzoic acid[166a] to D-mannose; an 80% yield of the phenylhydrazone was obtained. D-Glucal reacts with phenylhydrazine to give an oily product; with benzylphenylhydrazine a crystalline 2-desoxy-D-glucose benzylphenylhydrazone is formed.

7. *Mechanism of Halogen Oxidation*

a. Role of the Oxidant. As indicated by their respective oxidation potentials (Table I), the order of increasing effectiveness of the halogens as oxidants should be iodine, bromine, chlorine. This should hold whether the halogen is present as free halogen, hypohalous acid or hypohalite ion. The halogen most often used is bromine, and since there has been little use of other oxidants except sodium hypoiodite, it is difficult

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(165) M. Gehrke and F. X. Aichner, *Ber.*, **60**, 918 (1927).

(166) E. Fischer, M. Bergmann and H. Schotte, *Ber.*, **53**, 509 (1920).

(166*a*) M. Bergmann and H. Schotte, *Ber.*, **54**, 440 (1921).

to show the influence of oxidation potentials. Little work has been done with more than one oxidant under comparable conditions, especially that of concentration. For example, where chlorine and bromine have been used separately with the same sugar, lower yields have always been reported for the former halogen. Yet chlorine is always introduced as a gas, bromine as a liquid, and no attempt has been made to use a solution of each at the same concentration. Again, when low yields of product have been reported, few attempts have been made to ascertain whether this is due to incomplete oxidation of the original sugar or due to further oxidation of the product to another and unisolated substance. Ochi[135] showed that the low yields of D-gluconic acid obtained by the action of bleaching powder on D-glucose were due to "over-oxidation," leading to the formation of oxalic and saccharic acids.

The influence of *p*H on the course of an oxidation is definite, affecting not only the velocity of the reaction, but the direction as well. The inhibiting effect of accumulated hydrobromic acid in bromine oxidations was shown by Bunzel and Matthews[93] and others; in this case, the velocity of the reaction was reduced. The influence on the direction of the reaction was shown by Lal and Mukherji[111]; in the electrolytic oxidation of D-glucose, too high an acidity led to the formation of saccharic acid. The production of the latter may have been either a secondary reaction starting from D-gluconic acid, or a primary reaction. In either case the desired primary reaction was obscured to a certain extent.

The course of the reaction can be determined not only by the amount of product formed but also by the change in concentration of the actual oxidant. It is often difficult to determine the true concentration of the oxidant, because of its transient nature, or to determine in what form the oxidant is reacting with the sugar. Bunzel and Matthews[93] studied the rate of reaction of bromine with D-glucose quite extensively and determined the specific reaction rates. The reaction rates were related to the bromine concentration and it was concluded that the free halogen, rather than hypobromous acid, was the oxidant.

The study of the effect of *p*H on the reaction was not too satisfactory. As discussed previously (page 151), the oxidation is inhibited by acids, especially by hydrochloric and hydrobromic acids. It was concluded that the hydrobromic acid formed in the reaction, or added as an inhibitor, repressed the formation of the negative D-glucose ion, $C_6H_{11}O_6^-$, so that the main oxidation was that of the cation $C_6H_{13}O_6^+$ after the initially neutral solution became acid. The increased hydrogen ion concentration drove reaction 27 to the left. The cation was considered to be

$$C_6H_{12}O_6 \rightarrow C_6H_{11}O_6^- + H^+ \tag{27}$$

$$C_6H_{12}O_6 + H_2O \rightarrow C_6H_{13}O_6^+ + OH^- \tag{28}$$

the active form of the sugar in both acid and alkaline solution; therefore in alkaline solution the formation of this ion would be repressed (reaction 28 shifted to the left) and a low yield of D-gluconic acid should be obtained. As this was not the case it was decided that D-gluconic acid might be derived from other sources as well.

The considerably greater effect of hydrobromic and hydrochloric acids in inhibiting the oxidation appears to involve more than just acidity and might be considered as a repression of the available oxidant. This was considered by Bunzel and Matthews.[93] If bromine is the oxidant, two reactions (29 and 30) might be involved. However, we are more

$$H^+ + Br^- + Br_2 \rightleftarrows H^+ + Br_3^- \qquad (29)$$

$$H^+ + Cl^- + Br_2 \rightleftarrows H^+ + ClBr_2^- \qquad (30)$$

concerned here with reactions whose equilibria normally favor the formation of free halogen than with reactions in which the formation of complex ions is favored (see equilibrium constant K_3 in equation 33, page 174).

For weakly acidic systems (pH 5–6) in which the accumulation of hydrobromic acid is prevented by buffering agents such as calcium carbonate or benzoic acid salts, more information is available. Isbell and Pigman[167] have made an extensive study of such systems, including a thorough consideration of the effect of the concentration of total bromine, free bromine, hypobromous acid and bromide ion on the velocity of the reaction. The results very definitely showed a direct correlation between free bromine concentration and the velocity of the oxidation. No such correlation could be found with hypobromous acid. The results are shown in Tables VII and VIII. The velocity constants were determined for α- and for β-D-glucose. In the table for β-D-glucose, in experiments 2 and 5, the hypobromous acid concentration varied 1:10 but the reaction rate varied 1:3. The variations in free bromine concentration follow the variations in the reaction rate constants and the k_β values are based on the assumption that free bromine is the oxidant. The concentration of the oxidant (a in equation 31) is therefore the concentration of free bromine.

The lack of correlation between the concentration of hypobromous acid and the reaction velocity is shown very definitely in Table VIII for α-D-glucose. In the first three experiments the concentration of hypobromous acid shows an increase while the reaction velocity shows a decrease. In experiments 3, 4, and 5 the reaction velocity decreases with decreasing hypobromous acid concentration. In experiments 5 and 6 the hypobromous acid concentration increases greatly but the reaction

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(167) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards,* **10,** 337 (1933).

velocity shows only a slight increase. This lack of correlation between the concentration of hypobromous acid and the reaction velocity rules out any possibility of hypobromous acid being considered as the oxidant under these conditions. In Table VII, the evidence is not so definite.

TABLE VII

Correlation of Rate of Oxidation of β-D-Glucose with Concentration of Free Bromine[167]

Experiment	*β-D-Glucose*	*B, total bromide*	*C, total bromine*	*Free bromine (calcd.)*	*Hypobromous acid (calcd.)*	*Oxidation (%) after*			ak_β	k_β
						5 min.	*10 min.*	*15 min.*		
	Moles per liter	*Equivalents per liter*	*Moles per liter*	*Moles per liter*	*Moles per liter* $\times 10^{-4}$					
1	0.0496	0.412	0.351	0.0692	0.612	62.5	86.7	95.0	0.0873	1.26
2	0.0496	0.238	0.179	0.0477	0.580	56.7	80.9	91.0	0.0714	1.50
3	0.0496	0.564	0.186	0.0196	0.069	26.1	47.4	61.5	0.0272	1.39
4	0.0496	0.564	0.182	0.0192	0.068	26.8	47.0	60.5	0.0272	1.42
5	0.0496	0.560	0.176	0.0182	0.064	24.7	44.2	59.3	0.0253	1.39
									Average	1.39

TABLE VIII

Correlation of Rate of Oxidation of α-D-Glucose with Concentration of Free Bromine[167]

Experiment	*α-D-Glucose*	*B, total bromide*	*C, total bromine*	*Free bromine (calcd.)*	*Hypobromous acid (calcd.)*	*Oxidation (%) after*			$k_1 + ak$	ak_α	k_α
						30 min.	*60 min.*	*120 min.*			
	Moles per liter	*Equivalents per liter*	*Moles per liter*	*Moles per liter*	*Moles per liter* $\times 10^{-4}$						
1	0.0496	0.555	0.56	0.101	0.88	18.9	33.1	52.2	0.00287	0.00235	0.023
2	0.0496	0.381	0.374	0.085	0.94	16.6	28.8	49.1	0.00254	0.00202	0.024
3	0.0496	0.195	0.19	0.063	1.11	15.2	27.5	46.0	0.00231	0.00179	0.028
4	0.0496	0.550	0.365	0.051	0.28	12.9	25.8	39.8	0.0020	0.00148	0.029
5	0.0496	0.544	0.19	0.021	0.08	5.8	15.3	30.6	0.00113	0.00061	0.029
6	0.0985	0.390	0.37	0.081	0.85	15.4	29.0	45.4	0.00236	0.00184	0.023
										Average	0.026

A correlation of rate with hypobromous acid concentration exists, but a better one is shown for the concentration of free bromine.

The oxidations were carried out with a large excess of bromine to ensure a monomolecular reaction. The velocity constant was calculated by means of equation 31

$$ak = \frac{1}{t}\log\left(\frac{A}{A - x}\right) \tag{31}$$

where a = concentration of oxidant, t = time (in minutes), A = concentration of sugar at $t = 0$, and x = quantity of sugar oxidized in the time interval t. The data in Table IX show that the reaction proceeded at a fairly uniform rate and that the β-D-glucose was homogeneous, and not a mixture of two isomers.

TABLE IX

Oxidation of β-D-Glucose[a] *with Bromine at 0.3°C. ± 0.3°*[167]

Time, min.	*Unoxidized sugar, g.*	*Oxidized sugar, %*	ak_β	k_β
1.3	3.495	22.4	—	—
3.3	2.330	48.2	0.0878	—
5.1	1.655	63.2	0.0853	—
8.3	0.837	81.4	0.0886	—
11.1	0.483	89.3	0.0878	—
15.5	0.204	95.5	0.0871	—
25.3	0.080	98.2	(0.0681)	—
		Average	0.0873	1.26

[a] Crystalline β-D-glucose (4.5 g.) was added to a mixture of 500 cc. of barium bromide solution (containing 30 g. of $BaBr_2 \cdot 2H_2O$ and saturated with carbon dioxide), 10 cc. of bromine and 30 g. of barium carbonate. The average bromine concentration, as determined by titration with standard sodium thiosulfate, was 0.351 moles per liter, the average bromide content was 0.412 moles per liter and the concentration of free bromine was 0.0692 moles per liter.

The effect of variations in the concentration of the various possible oxidants was then studied. These possible oxidants are total bromine, free bromine and hypobromous acid. As no data were available regarding the concentration of free bromine in barium bromide solutions, formulas derived by Jones and Hartmann[168] for the bromine-potassium bromide system were applied, with the understanding that the values obtained would be only approximate.

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(168) G. Jones and M. L. Hartmann, *Trans. Am. Electrochem. Soc.*, **30**, 295 (1916).

The following equations and equilibrium constants were considered:

$$Br_2 + H_2O \rightleftarrows HOBr + Br^- + H^+$$

$$K_H = \frac{[H^+][Br^-][HOBr]}{[Br_2]} = 5.7 \times 10^{-10} \tag{32}$$

$$Br_2 + Br^- \rightleftarrows Br_3^-$$

$$K_3 = \frac{[Br^-][Br_2]}{[Br_3^-]} = 0.051 \tag{33}$$

$$2Br_2 + Br^- \rightleftarrows Br_5^-$$

$$K_5 = [Br^-][Br_2]^2/[Br_5^-] = 0.0246 \tag{34}$$

$$B = [Br^-] + [Br_3^-] + [Br_5^-] \tag{35}$$

$$C = [Br_2] + [Br_3^-] + 2[Br_5^-] + [HOBr] \tag{36}$$

The symbols represent the concentrations of the various ions and molecules in the equilibrium solution. The concentrations of free bromine and hypobromous acid were obtained by solving Jones and Hartmann's equations simultaneously and substituting the experimental values for the total bromine (C), total bromide (B), and hydrogen-ion concentration $[H^+]$. As the concentration of hypobromous acid was extremely small, it was neglected in equation 36. Equation 37 was obtained by substituting in equation 35 the value of $[Br_3^-]$ obtained from equation 33 and the value of $[Br_5^-]$ obtained from equation 34 and then solving for $[Br^-]$.

$$[Br^-] = \frac{B}{1 + \frac{[Br_2]}{0.051} + \frac{[Br_2]^2}{0.0246}} \tag{37}$$

Equation 38 was obtained by eliminating $[Br_5^-]$ from equations 35 and 36.

$$2B - C = 2[Br^-] - [Br_2] + [Br_3^-] \tag{38}$$

By substituting in equation 38 values of $[Br_3^-]$ and $[Br^-]$ in terms of $[Br_2]$ as obtained from equations 33 and 37, respectively, and simplifying, equation 39 was obtained. This equation was used for determining the concentration of free bromine.

$$[Br_2]^3 + (0.482 + 2B - C)[Br_2]^2 + [0.0246 + 0.482(B - C)]\,[Br_2] - 0.0246 = 0 \tag{39}$$

Since the value of $[Br_2]$ was small, the equation was easily solved by the method of successive approximations. In a preliminary calculation $[Br_2]^3$ was neglected and an approximate value of $[Br_2]$ was obtained. The cube of the value thus obtained was then substituted in equation 39 and the resulting quadratic equation again solved for $[Br_2]$. After repeating this process several times, the numerical value of $[Br_2]$ approached a constant which was the correct root of the equation.

The concentration of hypobromous acid was calculated from the concentrations of free bromine $[Br_2]$, total bromide (B) and total bromine (C) by means of equation 40 which was obtained by substituting in equation 38 the value of $[Br_3^-]$ from equation 33 and the value of $[Br^-]$ in terms of $[H^+]$, [HOBr] and $[Br_2]$ obtained from equation 32. The numerical value of $[H^+]$ is 4×10^{-6}.

$$[HOBr] = \frac{28 \times 10^{-4}(0.102 + [Br_2])[Br_2]}{2B - C + [Br_2]} \tag{40}$$

In this work and in a later paper,[169] Isbell and Pigman have very definitely shown the correlation between free bromine concentration and the velocity constant. Thus, in studies of the oxidation of twenty different sugars, the maximum deviation from the average value found for the velocity constants was less than 20% in most cases.

The question of the nature of the oxidant in an aqueous buffered bromine solution at *p*H 5–6 appears to be settled for the aldoses. But the same cannot be said for oxidation with bromine in a strongly acid solution in which hydrobromic acid is allowed to accumulate. The data of Isbell and Pigman show that an increase in bromide concentration has little effect on the velocity of the reaction. Yet Bunzel and Matthews[93] have shown that the addition of hydrobromic acid has a very definite inhibiting effect on the oxidation. Apparently, the combination of hydrogen ion and bromide ion has a pronounced effect, whereas either one alone is of minor importance.

b. Role of the Sugar. In the oxidation of various sugars, the influence of the structure and configuration of the sugar depends to a large extent on the experimental conditions; the sugar may exist in the reaction mixture in the aldehydo form or in the ring form, and in alkaline solution a certain amount of enolization may occur. Sometimes the nature of the reaction products is such that conclusions can be drawn regarding the role of the carbohydrate in the oxidation.

Isbell and Hudson[170] identified lactones as the oxidation products of aldoses in buffered solution. The conditions were such that any free aldonic acid formed would be converted into the salt. Hence, lactones are the initial oxidation products and it was concluded that "cyclic sugars give δ-lactones by oxidation with bromine water." The reactions were carried out in a solution buffered with barium carbonate and carbon dioxide. In the slightly acidic medium employed, the oxidation was rapid and interconversion between the anomeric forms of the sugars was relatively slow. In some cases the oxidation was 95% complete in five minutes. The extent of the reactions can be seen in Table X.

The products were δ-lactones and their concentration was determined by titration of the filtered solutions with alkali. Any free aldonic acid formed in the oxidation would be present in the solution as the barium salt and thus would not enter into this titration. The type of lactone was determined by the rate of change in the *p*H and in the optical rotation of the reaction solution, after the oxidation had been stopped by removal

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(169) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

(170) H. S. Isbell and C. S. Hudson, *J. Research Natl. Bur. Standards*, **8**, 327 (1932).

of the excess bromine. The more rapid hydrolysis of the δ-lactones, in comparison with the γ-lactones, permitted their identification.

This method was of value in that it gave an insight into the actual reaction of an aldose during bromine oxidation, and was also indicative of its ring structure. The method should be used with caution, however, especially in respect to the relative velocity of the anomeric forms of the various sugars. Formation of δ-lactones can be considered excellent evidence that the original aldose existed in the pyranose form in solution and reacted as such. Isbell and Frush[171] have applied this method in determining the structure of D-mannuronic lactone. Bromine oxidation gave a 1,4:3,6-dilactone, which was considered conclusive proof that the lactol ring in the original substance was 1,4.

TABLE X

Oxidation of Sugar Solutions at 0°C. with Bromine Water in the Presence of Barium Carbonate[169]

Sugar	*Composition of equilibrium solution*				*Rate of oxidation with bromine water at 0°C.*			
	Estimated from oxidation measurements		*Calculated from optical rotations, assuming only two constituents*		*Obtained with equilibrium solutions at 0.3°C.*		*Obtained with crystalline sugars*	
	Less reactive sugar, %	*More reactive sugar, %*	*α-Sugar, %*	*β-Sugar, %*	$k_\alpha \times 10^3$	$k_\beta \times 10^3$	$k_\alpha \times 10^3$	$k_\beta \times 10^3$
D-Glucose	37.4	62.6	36.2	63.8	27.5	1,362	32.4	1,255
D-Mannose	68.9	31.1	68.8	31.2	45.2	860	51.1	781
D-Galactose	31.4	68.6	29.6	70.4	37.9	1,720	42.3	1,590
D-Talose	55.9	44.1	—	—	84.8	844	78.5	—
(D-Gulose)$_2$ CaCl$_2$·H$_2$O	18.5	81.5	—	—	52.6	418	54.8	328
L-Arabinose	32.4	67.6	26.5	73.5[a]	84.2	1,608[a]	95.3	1,658[a]
D-Xylose	32.1	67.9	34.8[b]	65.2[b]	80.3	1,673	89.9	—
D-Lyxose	79.7	20.3	76.0	24.0	189	717	156	449
D-Ribose	89.3	10.7	—	—	180	1,010	196	—
L-Ribose	89.0	11.0	—	—	170	1,456	195	—
L-Rhamnose	69.0	31.0	73.1[b]	26.9[b]	83.4	770	89.7	—
Lactose	37.5	62.5	36.8	63.2	20.9	1,475	29.3	952
Maltose	37.7	62.3	36.0[b]	64.0[b]	23.7	1,388	—	1,528

[a] By the nomenclature of Hudson, these are the α-isomers.

[b] Percentage calculated from Hudson's optical rotations derived from measurements of the initial and final solubilities at 20°C. [C. S. Hudson, *Bur. Standards, Sci. Paper*, No. 533, 267 (1926)].

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(171) H. S. Isbell and Harriet L. Frush, *J. Research Natl. Bur. Standards*, **37**, 43 (1946).

In alkaline solution, enolization of the sugar can occur and may either facilitate or inhibit oxidation. Goebel[123] pointed out that D-glucose was slowly converted to D-fructose in alkaline hypoiodite solution and that the ketose was only slowly oxidized by the iodine. The bromine oxidation of D-glucosamine in acid solution is very slow, even slower than that of ketoses, and the effect is more pronounced with D-glucosamine hydrochloride than with the hydrobromide.[52] Here the inhibiting effect of the halogen halides is the reverse of that observed by Bunzel and Matthews[93] for D-glucose (see page 151).

An exception to the normal behavior of ketoses with alkaline sodium hypoiodite has been noted in the case of D-xyloketose.[171a] This ketopentose reduces very strongly and could not be distinguished in a mixture with D-xylose by the Willstätter-Schudel method.

c. Use of Catalysts. The use of catalysts has been investigated very little. In the few cases where such accelerators have been used, claims have been made for a beneficial effect, but control experiments without the catalysts have seldom been reported. Den Otter[155] noted that alkaline sodium hypochlorite would not oxidize glycerol in the absence of a trace of cobalt acetate; in neutral solution the reaction did not occur at all. Cobalt nitrate was used by Ling and Nanji[98] in the buffered bromine oxidation of D-glucose to D-gluconic acid. Here direct sunlight was also used to accelerate the reaction. Hönig and Ruzicka[137] used ultraviolet rays to increase the action of alkaline barium hypobromite or hypochlorite on D-glucose while Hönig and Tempus[139] used sunlight in the oxidation of D-glucose with alkaline hypobromite. In contrast, other workers, as for example, Hudson and Isbell[99] or Hart and Everett[69] shielded their reactions carefully from light.

Hypochlorous acid has been reported by Bonnet[11] to be unstable in the presence of visible light but only slightly affected by ultraviolet light. Sodium hypochlorite is attacked very rapidly by visible light. Bleaching powder is decomposed by cobalt salts with the liberation of oxygen. Apparently two factors are of importance. First, a photochemical effect due either to visible or ultraviolet light may influence the velocity or nature of the reaction. In the second place, decomposition of the oxidant may either lessen its activity toward the sugar, or by liberation of oxygen cause an oxidation of a type different than that anticipated.

Inorganic bromides have been termed catalysts in indirect oxidations —those in which the solution is treated with chlorine or oxidized electrolytically; in this type of reaction the bromide is converted to free bromine and then reconverted into bromide.

(171*a*) O. T. Schmidt and R. Treiber, *Ber.*, **66**, 1765 (1933).

III. OXIDATION WITH HALOGEN ACIDS IN ACID SOLUTION

Both chloric and iodic acid have been used as oxidants in acidic solutions. No examples with bromic acid have been reported. With aldonic acids the primary products are keto acids. However, an example does exist of the conversion of an aldose to an aldonic acid. This might be classed as an indirect or secondary bromine oxidation, since a mixture of sodium chlorate and hydrobromic acid was used in acid solution.

Both chlorates and iodates have been used with catalysts, primarily vanadium pentoxide. Pasternack and Regna[172] have prepared 2-keto aldonic acids by the oxidation of aldonic lactones or aldonic acid salts with sodium chlorate and a vanadium catalyst in mildly acid solution. Regna and Caldwell[173] prepared methyl 2-keto-D-gluconate and methyl 2-keto-D-galactonate by oxidation of 1,4-D-gluconic lactone and potassium D-galactonate, respectively, in methanol solution acidified with phosphoric acid, using sodium chlorate with vanadium pentoxide as the oxidant. A 60% yield of the keto-D-gluconate and a 30% yield of the keto-D-galactonate resulted after two to three days at 30°.

More drastic conditions were used with iodic acid, high temperatures and strongly acid solutions being employed. The oxidation products were not isolated. Williams and Woods[174] used 35% sulfuric acid containing 1% potassium iodate, the sugar being heated for ninety minutes at 100° with this oxidant. Ketoses, sucrose and pentoses were attacked, but L-rhamnose was oxidized more slowly. It was remarkable that none of the aldohexoses or lactose were detectably oxidized. The reactions of cellobiose and trehalose were ascribed to impurities.

Still higher temperatures and longer periods of time were used by Hurka[175]; at 130° and eighteen to twenty-one hours, D-glucose, D-galactose, D-mannose and D-fructose were quantitatively oxidized to carbon dioxide and water. D-Fructose initially reacted more rapidly than the aldoses but there was little difference in the rate after ninety minutes, when 5 atoms of oxygen had been consumed per mole of sugar. D-Fructose reacted with 4 atoms of oxygen in five minutes, and D-mannose reacted almost as quickly.

Under mild conditions neither ketoses nor aldoses are attacked by chloric acid or by chlorates. Jeanes and Isbell[176] used 0.15 *M* potassium chlorate in water and in acid solutions (0.56 *M* with acetic and phosphoric

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(172) R. Pasternack and P. P. Regna, U. S. Pats.: 2,203,923; 2,207,991; 2,188,777; 2,222,155 (1940).

(173) P. P. Regna and B. P. Caldwell, *J. Am. Chem. Soc.*, **66**, 243 (1944).

(174) R. J. Williams and M. A. Woods, *J. Am. Chem. Soc.*, **59**, 1408 (1937).

(175) W. Hurka, *Mikrochemie ver. Mikcrochim. Acta*, **30**, 259 (1942).

(176) Allene Jeanes and H. S. Isbell, *J. Research Natl. Bur. Standards*, **27**, 125 (1941).

acid) and found no change for D-glucose, D-fructose, L-arabinose or sucrose in fourteen days. Alkaline sodium bromate at temperatures ranging from 20 to 70° for twenty-four hours had no effect on D-glucose, sucrose or cellulose.[177]

IV. OXIDATION WITH CHLOROUS ACID AND THE CHLORITES

The recent commercial production of sodium chlorite[178] has made this oxidant available, and its ability to oxidize lignin in wood without attacking the carbohydrate portion has aroused special interest. Investigation of this reagent with the simple sugars under various conditions is very interesting. Sucrose was completely inert under non-hydrolytic conditions. The ketoses, aldonic acids and polyhydroxy alcohols were attacked only very slowly. Aldoses were attacked and the products isolated were aldonic acids and a trace of oxalic acid. Jeanes and Isbell[176] have found that the action is more rapid with pentoses than with hexoses, as is also the case for iodic acid. Monosaccharides are attacked more rapidly than disaccharides. In the case of D-glucose, the α-form is oxidized slightly faster than the β-form; this effect is the reverse of that observed when bromine is the oxidant (page 182).

The yields of aldonic and oxalic acids are shown in Table XI. They are based on the amount of sugar oxidized (determined by reducing value) and not on the total amount of starting material. The formation of L-arabonic acid occurs with the highest percentage yield, but even this is low compared with other methods of preparation. As the lactobionic acid was isolated as the calcium lactobionate-calcium bromide salt, the actual amount of product, expressed in millimoles, is very low.

An attempt was made by Jeanes and Isbell[176] to evaluate their experimental data (Table XII) and to determine the mechanism of the reaction of chlorites with aldoses. Two of the experiments were carried out in acid solution at 0° and 20°, and the third in neutral media, as with the experiments in Table XI. Reaction 41 was believed to express the course of the oxidation in highly acid solution. The deviations, as expressed in higher consumption of chlorous acid and in higher yields of chloride, are

$$\mathrm{RCHO + 3HClO_2 \rightarrow RCOOH + HCl + 2ClO_2 + H_2O} \tag{41}$$

$$\mathrm{3HClO_2 \rightarrow HCl + 2HClO_3} \tag{42}$$

$$\mathrm{2RCHO + HClO_2 \rightarrow 2RCOOH + HCl} \tag{43}$$

$$\mathrm{2ClO_2 + H_2O \rightarrow HClO_2 + HClO_3} \tag{44}$$

due to the conversion of chlorous acid to chloric acid and hydrogen chloride (reaction 42). Since chlorine dioxide is found and since reaction 43 does not allow for its formation, this reaction can probably be ignored.

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(177) P. Van Fossen and E. Pacsu, *Textile Research J.*, **16**, 163 (1946).

(178) M. C. Taylor, J. F. White, G. P. Vincent and G. L. Cunningham, *Ind. Eng. Chem.*, **32**, 899 (1940).

In neutral solution very little chlorate should be produced, since chlorites in aqueous solution are stable in the absence of reducing agents. However Experiment III in Table XII does not bear out this conclusion.

TABLE XI

Oxidation of Aldoses in Neutral Chlorite Solution

(Calculated by the author from data of Jeanes and Isbell[176])

Aldose	*Initial amount of aldose*		*Amount of aldose oxidized*		*Ca aldonate isolated*		*Per cent of theory*	*Ca oxalate isolated*		*Per cent of theory*
	g.	*moles*	*g.*	*moles*	*g.*	*moles*		*g.*	*moles*	
D-Glucose	25.0	0.139	17.0	0.094	3.1	0.014	15.3	0.49	0.004	4.0
L-Arabinose	21.0	0.141	19.5	0.130	12.9	0.069	53.5	0.77	0.006	4.6
Lactose	48.0	0.140	30.5	0.088	8.3	0.001	0.9	0.12	0 001	1.1

TABLE XII

Quantitative Study of the Products Formed by the Oxidation of D-*Glucose with Chlorites in Acid and in Neutral Solution*[176]

Constituents	*Experiment*			*Theory*		
				For reactions		*For Exp. III corrected for reaction 44*
	I[a]	*II*[b]	*III*[c]	*41*	*43*	
	Moles[d]			*Moles*[d]		
Chlorous acid consumed	3.83	3.19	2.59	3.0	0.5	3.10
Chloride formed	1.35	1.24	1.24	1.0	0.5	1.24
Chlorate formed	0.47	0.14	0.51	0.0	0.0	0.0
Chlorine dioxide formed	2.01	1.82	0.85	2.0	0.0	1.87
Oxalic acid formed	None	None	0.01	0 0	0.0	0.01

[a] 0.0555 moles of D-glucose, 0.1516 moles of $NaClO_2$ and 0.168 moles of acetic acid in 100 cc. of water at 0° for twenty-four hours.

[b] 0.0555 moles of D-glucose, 0.0531 moles of $NaClO_2$ and 0.056 moles of acetic acid in 100 cc. of water at 20° for twenty-four hours.

[c] 0.0555 moles of D-glucose and 0.0248 moles of $Ca(ClO_2)_2$ in 100 cc. of water and $CaCO_3$ at 20° for seven days.

[d] Per mole of D-glucose oxidized.

The amount of chlorate formed is more than that formed in acidic solution. Jeanes and Isbell explain this by postulating the decomposition of chlorine dioxide in water to form chlorous and chloric acids (reaction 44). The amount of chlorate found in Experiment III would represent the decomposition of 1.02 moles of chlorine dioxide; this amount added to the 0.85 moles actually found would give 1.87 moles of chlorine dioxide formed, a value which compares favorably with those for the other two experiments and with the theoretical value for reaction 41. The hydrolysis of this additional 1.02 moles of chlorine dioxide would produce 0.51 moles of chlorous acid. As the amount of chlorous acid consumed was determined by the difference between the initial and final solutions, the concentration of the final solution should be corrected for this additional amount of chlorous acid. This would raise the total amount of oxidant consumed from 2.59 to 3.10 moles. These corrected values for Experiment III are given in the last column of Table XII.

These data are very interesting as a possible explanation of the course of the oxidation. However, the amounts of the products actually isolated (Table XI) do not support a straight-forward mechanism such as reaction 41. If aldonic acids are the only products, then high yields should be expected. This was apparently not the case, and no attempt has been made to explain this discrepancy.

V. EFFECT OF STRUCTURE OF THE SUGARS ON THE COURSE OF OXIDATION

In their speed of reaction with the halogens, in acid or in alkaline solutions, the simple sugars may be divided into two main classes, the aldoses and the ketoses. The oxidation of the former is very rapid compared with that of the latter. Kiliani[28] showed that when D-glucose and D-fructose were each treated with an equal weight of bromine in water, the ketose required 350–500 hours for completion of the oxidation, in contrast to two to three hours for the aldose. The same difference exists in buffered solutions; Hönig and Ruzicka[94] found that the rates were two hours and five minutes, respectively. In alkaline solution under controlled conditions, the aldoses can be quantitatively oxidized with sodium hypoiodite in the presence of D-fructose or L-sorbose without appreciable attack on the ketoses. Ochi[135] reported a similar but less clear-cut difference with calcium hypochlorite as the oxidant. Chlorous acid attacks only the aldoses, leaving the ketoses unaltered. The same effect was noted with the keto acids; Kiliani[82] reported that 2-keto-L-rhamnonic acid was stable to the action of bromine water. A little preliminary work has been done with iodic acid by Williams and Woods[174] who found that D-fructose was oxidized more rapidly than the aldoses. No confirmation of this work has appeared.

Within the aldose series, differences in rate of oxidation have been shown. Isbell and Pigman[179] found that a buffered bromine solution

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(179) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

attacked pentoses most rapidly; hexoses were oxidized more slowly and the disaccharides still more slowly. L-Rhamnose was oxidized at a rate similar to that for the pentoses. These rates are shown in Table X. The rates of oxidation in alkaline sodium hypoiodite solution were investigated by Myrbäck[125,180]; β-D-glucose was oxidized more rapidly than β-D-mannose, and maltose was oxidized at a slower rate than the hexoses. Steric hindrance arising from the *cis* position of the hydroxyl on the second carbon atom in relation to the hydroxyl group on the first carbon atom was assumed to have an important effect for the hexoses.

Very extensive work was done by Isbell and Pigman[100] on the rate of bromine oxidation of the anomeric forms of various aldoses in buffered solution. Except for arabinose, the β-forms were oxidized the most readily (Table X and Fig. 1). It is interesting to note that the rates for the two anomeric forms of a sugar are different, so that the rates can be used to determine the relative amounts of the two forms present in an equilibrium mixture. Thus for D-glucose the ratio of 64% β-D-glucose to 34% α-D-glucose was found; rotation data gave 65 and 35%, respectively. This is a very definite check on the assumption that the equilibrium mixture of this sugar consists essentially of only two forms. In the case of some other sugars, such as D-galactose, L-arabinose, D-talose and D-ribose, the possibility of the existence of more than two forms in solution is not excluded.

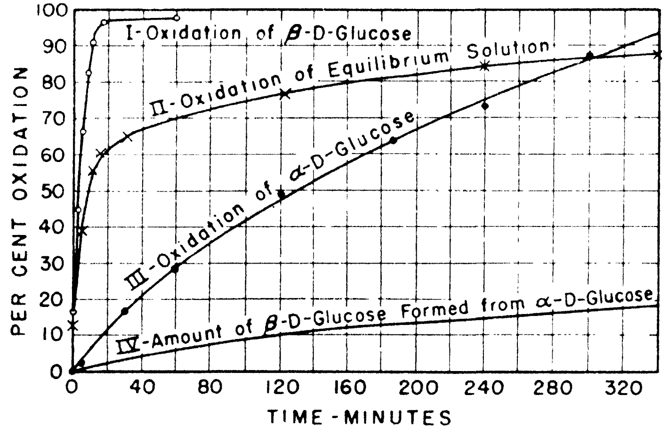

FIG. 1.—Bromine oxidation of D-glucose in the presence of barium carbonate.[167]

In contrast to the action of bromine, chlorous acid was found by Jeanes and Isbell[176] to oxidize α-D-glucose at a slightly higher rate than β-D-glucose (see Fig. 2).

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(180) K. Myrbäck, *Svensk Kem. Tid.*, **52**, 293 (1940).

Normally the rate of oxidation of D-glucose by hypoiodite is independent of the type of buffer. However, with borate buffers[126] the rate is decreased to 5% of the normal value. This effect is attributed to complex formation in the 1,2-position, since the rates of oxidation of 2,3,4,6-tetramethyl- and 2,3,6-trimethyl-D-glucose are approximately the same in borate buffers as in buffers of other types. These rates are about two-thirds of those for D-glucose in normal buffers. The effect of borate as a buffer has been confirmed by other workers. The original Romijn[121] method used a borate buffer and the reaction was found to be too slow for a convenient analytical method. Pacsu[20] found the same effect in the analysis of oxidized cellulose. The hypoiodite reagent was more stable in such a buffer but the velocity of reaction was very low.

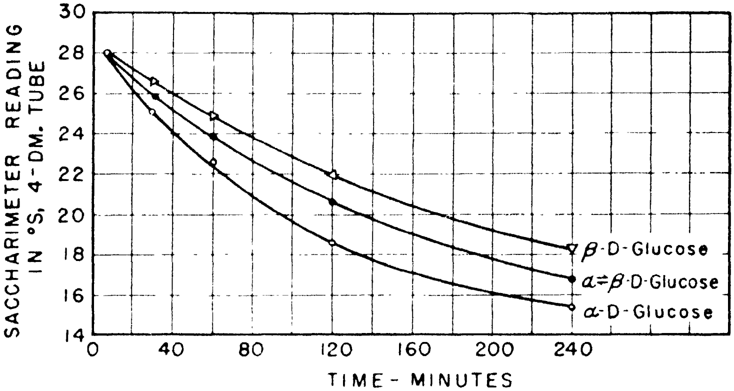

FIG. 2.—The oxidation of the α and β modifications of D-glucose by chlorous acid at 0°C.[176] The reaction solutions were prepared at 0°C. from 0.056 mole of the sugar, 0.167 mole of sodium chlorite, and 0.167 mole of acetic acid in sufficient water to give 100 ml. at 0°C. At intervals, 12-ml. samples were removed and added to equal volumes of 3 *M* sodium bisulfite solution at 0°C. The temperature was allowed to rise to 20°C. and after 24 hours the optical rotation of the sample was read in a 4-dm. tube.

VI. CONCLUSION

The field of halogen oxidation has developed primarily in two directions. The first is the preparation of aldonic acids by oxidation with bromine in acid solution. The second is the use of alkaline hypoiodite as a quantitative oxidant. Little work has been done on the mechanism of these reactions. The conditions under which the reactions are carried out have usually been established empirically without extensive study. The further use of solutions of known concentration and the comparison of various oxidants under similar conditions are to be desired. Critical oxidation potentials of various sugars used in relation to possible oxidants and ranges of *p*H might yield promising results.

It is surprising that chlorine has been so little investigated as an oxidant for sugars. Its cheapness recommends it from one point of view, its stability from another; in contrast to other halogens, it does not tend to form halogenates, and its use would thus appear to be desirable for fundamental work in which an accurate determination of concentration is necessary. Perhaps its action is more drastic than that of bromine or iodine. Two commercial methods in which it is used in conjunction with bromide salts as catalysts imply that bromine may be the best general oxidant.

The widespread use of bleaching agents containing chlorine, and of the halogens themselves as modifying agents for starch and cellulose should be based on a more fundamental study of the reactions involved.

THE MOLECULAR CONSTITUTION OF CELLULOSE

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I. Introduction

The molecular constitution of cellulose as elucidated by the classical researches of the Haworth school in 1926, has, since that time, been the basis of the interpretation of the chemical and much of the physical behavior of this principal plant cell wall constituent. The enthusiasm of the advocates of the theory stating that cellulose consists of long uniform chains of anhydro-D-glucose units has, during the past two decades, so completely dominated thinking in the field that there has been little encouragement for the establishment of any other supplementary concept. Within recent years, however, considerable evidence has accumulated which indicates the inadequacy of certain ideas which have become accepted as conventional with respect to the structure of purified cellulosic materials. In view of the changing concept of the structure of the cellulose molecule it is well to review the foundations on which the conventional ideas are based and determine if the more recent approaches to the problem may be incorporated in, and become a part of, a broadened concept.

II. Definitions

The heterogeneity of naturally occurring plant cell wall membranes in physical and chemical composition has been repeatedly demonstrated and in many cases is quite apparent upon casual visual inspection. The principal constituent of most growing plants, however, is a skeletal material which was first named "cellulose" as a result of the researches of Payen.[1] The difference in the composition of various plants was thought to be due to differences in the amount and nature of the "incrusting substances" associated with the cellulose component. The empirical formula of the isolated cellulose on the basis of modern atomic weights was found to be $C_6H_{10}O_5$[2,3] and was thought to be invariably composed of D-glucose residues. Thus, cellulose in the Payen sense is a definite carbohydrate which occurs abundantly in plants as a skeletal substance which on complete hydrolysis yields only D-glucose.

The early observation that many of the plant materials purified by the treatments described by Payen were composed of other carbohydrates, led to the rise of the second school of thought concerning the definition of cellulose.[4,5,6,7] This concept, expressed by Cross and Bevan in many publications,[8] stated that the term "cellulose" did not denote a

(1) A. Payen, *Compt. rend.*, **7**, 1052, 1125 (1838); **8**, 169 (1839); **9**, 149 (1839). A committee of the French Academy, with J. B. Dumas as chairman, made a report concerning Payen's researches on the composition of woody material and proposed the name "cellulose," *Compt. rend.* **8**, 51 (1839). This word also occurs in Payen's third article cited above.

(2) M. Phillips, *J. Wash. Acad. Sci.*, **30**, 65 (1940).

(3) J. D. Reid and E. C. Dryden, *Textile Colorist*, **62**, 43 (1940).

(4) R. Reiss, *Ber.*, **22**, 609 (1889).

(5) E. Schulze, *Ber.*, **24**, 2277 (1891); *Z. physiol. Chem.*, **16**, 387 (1892).

(6) E. Fremy, *Compt. rend.*, **48**, 275 (1859); *J. pharm. chim.*, [3] **36**, 5 (1859).

(7) J. Erdmann, *Ann.*, **5**, Suppl., 223 (1867).

(8) E. J. Bevan and C. F. Cross, *J. Chem. Soc.*, **38**, 666 (1880); C. F. Cross and E. J. Bevan, **41**, 90 (1882); **43**, 18 (1883); **55**, 199 (1889). C. F. Cross and E. J. Bevan, "Cellulose," 1st. ed. (1895); 2d ed. (1903); 3d. ed. (1916); "Researches on Cellulose," 1895–1900; 1900–1905; 1905–1910; Longmans Green and Co., London.

definite chemical entity but rather was an expression designating the fibrous aggregate remaining after various chemical treatments were carried to the extent accepted as giving "pure" cellulose. Thus, in the plant it was believed that the "cellulose" and "incrusting materials" of Payen, merged into one another by indetectable chemical gradations. The "incrusting materials" included such plant materials as hemicelluloses, pectin, lignin and fatty materials. The complexes existing preformed in the plant were designated by the prefixes in such words as, "ligno-," "pecto-" or "muco-," and "cuto-cellulose," according to the constituent predominating. The terminology of Cross and Bevan is now considered obsolete, but the fundamental idea that cellulose is not a homogeneous chemical compound independent of plant source has found continued support.

At the present time it appears doubtful that cellulose in the Payen sense has ever been isolated although there is a possibility that this may be accomplished. On the other hand, cellulose in the Cross and Bevan sense is the product resulting from the "purification" of plant materials, such as wood and cotton linters or fibers, by rather empirical procedures. As shown by Sherrard and Blanco,[9] alkali-purified wood pulps yield D-mannose on hydrolysis. Special methods have been devised which reduce the xylan and mannan content to a minimum but the persistence of these materials is certain.[10] The alternate chlorination and sodium sulfite extraction procedure for determining the cellulose content of plant materials by the Cross and Bevan procedure[8] yields a fibrous product containing cellulose and cellulosans. Subsequent extraction of Cross and Bevan cellulose with alkali results in the removal of the greater part of the cellulosans. The concept of the cellulosans and their relationship to the cellulose component rests on the Cross and Bevan cellulose procedure. This procedure separates those polysaccharides which, because of their solubilities in dilute alkalies and acids are generally classified as hemicelluloses, from the cellulosans and the cellulosic residue, of which the cellulosans are a part. The polyuronide hemicelluloses which are soluble in the hot sulfite are similar to the hemicelluloses first described by Schulze[5] as being extracted from plant materials by dilute acids, whereas the cellulosans correspond to the alkali resistant fraction described by Hawley and Norman.[11]

Hemicelluloses have been defined by Norman[12] as those cell wall polysaccharides which may be extracted from plant tissues by treatment

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(9) E. C. Sherrard and G. W. Blanco, *J. Am. Chem. Soc.*, **45**, 1010 (1923).

(10) F. Olsen, *Ind. Eng. Chem.*, **30**, 524 (1938).

(11) L. F. Hawley and A. G. Norman, *Ind. Eng. Chem.*, **24**, 1190 (1932).

(12) A. G. Norman, "Biochemistry of Cellulose, Polyuronides, Lignin, etc.," p. 37, Oxford University Press, London (1937).

with dilute alkalies (either hot or cold) but not with water, and which may be hydrolyzed to constituent sugar and sugar acid units by boiling with hot dilute mineral acids. The relationship of the cellulosans to cellulose is claimed to be similar to that existing between the components of a solid solution or a mixed crystal.[13] Since it has been shown[14] that the cellulosans are simultaneously laid down with the supposed pure cellulose at all stages of plant growth and form an integral part of the skeletal structure, no sharp line of demarcation can be drawn between the structural and incrusting polysaccharides envisioned by Payen.

When plant tissues are treated with chlorine and subsequently with a hot 95% alcohol solution containing three per cent monoethanolamine, a residue is obtained containing the complete carbohydrate fraction of these materials and known as holocellulose.[15,16] This method serves as a very convenient means of obtaining plant materials lignin-free for determining the relationship existing between the various carbohydrate fractions.

Another method of isolating holocellulose from wood and similar lignified tissues by the use of sodium chlorite and acetic acid was devised by Jayme[16a] and modified by Wise, Murphy and D'Addieco.[16b] The latter method serves as a convenient means of obtaining plant materials nearly free from lignin and of determining quantitatively the relationship existing between various carbohydrates of the cell wall.

By the treatment of Cross and Bevan cellulose, or cellulose isolated by some other purification procedure, with 17.5% sodium hydroxide under carefully controlled conditions, and then carefully washing, a fibrous product is obtained which is termed α-cellulose.[8] As previously indicated, Cross and Bevan used this procedure for determining the purity of cellulose preparations. This product upon further similar treatments continues to lose weight, indicating the empirical nature of the treatment and terminology. Upon acidifying the alkaline filtrate a precipitate is formed. This fraction is known as β-cellulose. The alkali-soluble extract which remains in solution after acidification of the solution is termed γ-cellulose. Since continued extraction of α-cellulose with caustic causes the formation of increasing amounts of β-and γ-cellulose, the incomplete removal of these fractions in one operation or the conversion of α-cellulose into β- and γ-cellulose is indicated. In practice

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(13) W. T. Astbury, W. D. Preston and A. G. Norman, *Nature*, **136**, 391 (1935).

(14) A. G. Norman, *J. Agr. Sci.*, **23**, 216 (1933).

(15) W. G. Van Beckum and G. J. Ritter, *Paper Trade J.*, **105**, 127 (Oct. 28, 1937).

(16) Technical Association of the Pulp and Paper Industry, Tentative Standard T 9 M-40 (May 30, 1940).

(16a) G. Jayme, *Cellulosechem.* **20**, 43 (1942).

(16b) L. E. Wise, Maxine Murphy and A. A. D'Addieco, *Paper Trade J.*, **122**, 35 (Jan. 10, 1946).

both mechanisms are possible. However, although α-cellulose may be completely converted into β-cellulose, β-cellulose is not easily converted into a state which would classify it as γ-cellulose. It is now known that β-cellulose from carefully purified cellulosic materials is really α-cellulose of short chain length plus possibly some contaminating cellulosans, whereas γ-cellulose, although a short-chain polysaccharide, is distinguished by structural differences similar to that existing in the polyuronide hemicelluloses.[17,18]

Plant materials vary considerably in the proportion of cellulosans to cellulose. Cotton fiber is unique in consisting largely of long anhydro-D-glucose chains. After purification by treatment with dilute caustic and carefully washing, a product of extremely high α-cellulose content is obtained which has been used as a standard of purity in evaluating other cellulosic materials.[19] Thus, carefully purified cotton fiber or linters has been accepted by many as fulfilling the requirements of the term cellulose in the Payen sense. However, in view of the demonstrated complexity of the composition of the developing cotton fiber,[20] it is probable that an interrelationship exists between the various carbohydrate constituents which is similar to that present in the case of other plant cell-wall materials, but differing in that the cellulose fraction in the Payen sense predominates to a greater extent.

From these considerations a completely satisfactory chemical definition of cellulose cannot be given at present, but for the purposes of this discussion the term will be used in a plural sense somewhat more comprehensive than that employed by Payen. The term "cellulosic material" or "purified cellulosic material" will be used interchangeably with the term cellulose, but is meant to convey the same idea concerning composition.

III. CHAIN STRUCTURE OF CELLULOSE

1. D-*Glucose, the Basic Unit*

The marked uniformity and apparent homogenity of carefully purified cotton fiber has made this a desirable material for determining the structure of cellulose. The acetylation of purified cotton fiber was thus shown by Irvine and Soutar[21] to yield cellulose triacetate which upon treatment with methyl alcoholic hydrogen chloride gave a mixture of methyl α- and

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[17] L. E. Wise, editor, "Wood Chemistry," p. 599. Reinhold, New York (1944).

[18] R. L. Mitchell, *Ind. Eng. Chem.*, **38**, 843 (1946).

[19] C. Dorée, "The Methods of Cellulose Chemistry," p. 5. Van Nostrand, New York (1933).

[20] J. Compton and F. E. Haver, Jr., *Contrib. Boyce Thompson Inst.*, **11**, 105, 281 (1940); J. Compton, *ibid.*, **11**, 403 (1941).

[21] J. C. Irvine and C. W. Soutar, *J. Chem. Soc.*, **117**, 1489 (1920).

β-D-glucosides in 85% yield. Later refinement of this technique by Irvine and Hirst[22] resulted in 95.5% of the theoretical yield being obtained. Direct hydrolysis of purified cotton fiber with 72% sulfuric acid was shown by Monier-Williams[23] to yield 90.7% crystalline D-glucose. Although these researches are frequently cited as being decisive proof that purified cotton fiber consists entirely of D-glucose residues, the data does not guarantee the validity of this conclusion.

2. *Basic Unit Hydroxyl Groups*

The early studies of Ost[24] and Crum[25] indicated that the highest acetate and nitrate of purified cotton fiber was the triacetate and the trinitrate, respectively; this result showed that the basic unit of structure contained three hydroxyl groups. The position of the three hydroxyl groups could not be determined, however, until the discovery, by Denham and Woodhouse,[26] that cotton, when successively treated with caustic soda and dimethyl sulfate, slowly acquired methyl ether groups. The fibrous product thus obtained had a methoxyl content of 25% and upon hydrolysis yielded a crystalline trimethylglucose identical with 2,3,6-trimethyl-D-glucose. Irvine and Hirst,[27] using an improved technique, obtained a 70% yield of a methyl ether containing 42–43% methoxyl. The yield of crystalline 2,3,6-trimethyl-D-glucose obtained upon hydrolysis of this product corresponded to an over-all yield of 78.7%. Many later researches[28,29,30,31] still further improved the methylation technique until it is now possible to obtain methylated cellulosic fiber materials containing about 45% methoxyl in 90% yield in a few steps.

Although Denham and Woodhouse[26] found indications of the presence of a trace of tetramethyl-D-glucose among the hydrolysis products of their partially methylated cellulosic material, other investigators[28,29] were unsuccessful. Later, however, Haworth and Machemer[32] isolated tetramethyl-D-glucose from the hydrolyzate of a highly methylated cellulosic material containing 45% methoxyl. Comfirmation of these

(22) J. C. Irvine and E. L. Hirst, *J. Chem. Soc.*, **121**, 1585 (1922).

(23) G. W. Monier-Williams, *J. Chem. Soc.*, **119**, 803 (1921).

(24) H. Ost, *Z. angew. Chem.*, **19**, 993 (1906).

(25) W. Crum, *Phil. Mag.*, **30**, 426 (1847); *Ann.* **62**, 233 (1847).

(26) W. S. Denham and Hilda Woodhouse, *J. Chem. Soc.*, **103**, 1735 (1913); **105**, 2357 (1914); **111**, 244 (1917).

(27) J. C. Irvine and E. L. Hirst, *J. Chem. Soc.*, **123**, 518 (1923).

(28) K. Hess and W. Weltzien, *Ann.*, **442**, 49 (1925).

(29) K. Freudenberg and E. Brown, *Ann.*, **460**, 289 (1928).

(30) W. N. Haworth, E. L. Hirst and H. A. Thomas, *J. Chem. Soc.*, 821 (1931).

(31) K. Freudenberg, E. Plankenham and H. Boppel, *Ber.*, **71**, 2435 (1938).

(32) W. N. Haworth and H. Machemer, *J. Chem. Soc.*, 2270 (1932).

results soon followed.[33] However, Hess and coworkers[34] demonstrated that the quantities of tetramethyl-D-glucose obtainable from practically undegraded cellulosic material owed their formation to degradation during methylation. When methylation was conducted in a nitrogen atmosphere, the degradation was reduced to a minimum and no tetramethyl-D-glucose was found. This conclusion was immediately verified by the Haworth school,[35] but at the same time data were presented showing that, according to viscosity and osmotic pressure measurements, degradation had occurred. When methylation was conducted in the presence of oxygen, tetramethyl-D-glucose was again obtained.[36]

In addition to the esterification and etherification studies, the presence of three alcoholic hydroxyl groups has been shown by the action of metallic sodium in liquid ammonia on cellulosic materials.[37,38] For each D-glucose unit, one atom of sodium reacts readily while the two other atoms enter more slowly.

The results of these investigations thus indicate that: (a) there are one primary and two secondary hydroxyl groups in each basic anhydro-D-glucose unit ($C_6H_{10}O_5$), the hydroxyls being in positions 2, 3, and 6, respectively; (b) the end group of each long chain of anhydro-D-glucose units contains an additional secondary hydroxyl group in position 4; (c) positions 1, 4, and 5 in each anhydro-D-glucose unit are involved in some form of chemical linkage.

3. *Other Groups in the Molecular Structure*

a. Hemiacetal Groups. Although it is highly improbable that reducing groups are present in undegraded cotton and wood fiber,[39] yet as a result of more or less drastic purification treatments, degradation does occur, and reducing groups are found in samples of purified cotton linters and wood pulp. The presence of reducing groups in degraded cellulosic materials has been shown by: (a) the reduction of alkaline cupric copper

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(33) F. J. Averill and S. Peat, *J. Chem. Soc.*, 1244 (1938); E. L. Hirst and G. T. Young, *ibid.*, 1247 (1938).

(34) K. Hess and F. Neumann, *Ber.*, **70**, 710 (1937); K. Hess, D. Grigorescu, E. Steurer and H. Frahm, *ibid.*, **73**, 505 (1940); K. Hess and E. Steurer, *ibid.*, **73**, 669 (1940); K. Hess and K. P. Jung, *ibid.*, **73**, 980 (1940).

(35) W. N. Haworth, E. L. Hirst, L. N. Owen, S. Peat and F. J. Averill, *J. Chem. Soc.*, 1885 (1939); W. N. Haworth, R. E. Montonna and S. Peat, *ibid.*, 1899 (1939).

(36) W. N. Haworth, *Chemistry & Industry*, **17**, 917 (1939).

(37) P. C. Scherer, Jr., and R. E. Hussey, *J. Am. Chem. Soc.*, **53**, 2344 (1931).

(38) P. Schorigin and N. N. Makarowa-Semljanskaja, *Ber.*, **69**, 1713 (1936).

(39) A. M. Sookne and M. Harris, *J. Research Natl. Bur. Standards*, **26**, 205 (1941); *Am. Dyestuff Reptr.*, **30**, 107 (1941).

to the cuprous state (copper number)[40]; (b) the reduction of silver from the ionic to the metallic state[41]; (c) the condensation reaction with phenylhydrazine (phenylhydrazine number)[42,43,44]; (d) the condensation reaction with ethyl mercaptan[45]; (e) the reduction of alkaline hypoiodite solutions.[46] These methods have been used for quantitatively determining the potential reducing groups in cellulosic materials and, hence, the average chain length (degree of polymerization). They will be considered later in more detail.

b. Carboxyl Groups. The presence of acidic groups (undoubtedly carboxyl groups) in carefully purified cotton fibers has been shown by Schmidt and coworkers[47] using a conductometric procedure, and by Heymann and Rabinov,[48] as well as by Harris and coworkers[49] using ion exchange procedures. Whether this carboxyl group is present as a saccharic, uronic, or glyconic acid has not been determined, although the latter has been tacitly assumed since the carboxyl group has been considered as an end group in the cellulose molecule.

c. Unidentified Groups. The possibility of cross linkages existing between long anhydro-D-glucose chains has introduced a new viewpoint as to just what constitutes the cellulose molecule. According to Kleinert, Hingst and Simmler,[50] various alien groups, not necessarily carbohydrate in nature, may bind the anhydro-D-glucose chains together. The degradation of cellulosic fiber materials was postulated as being due

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(40) C. G. Schwalbe, *Ber.*, **40**, 1347 (1907); D. A. Clibbens and A. Geake, *J. Textile Inst.*, **15**, T27 (1924); J. O. Burton and R. H. Rasch, *Bur. Standards J. Research*, **6**, 603 (1931); S. M. Neale, *Chemistry & Industry*, **14**, 602 (1936).

(41) K. Götze, *Melliand Textilber.*, **8**, 624, 696 (1927).

(42) C. J. Staud and H. LeB. Gray, *Ind. Eng. Chem., Anal. Ed.*, **1**, 80 (1929).

(43) M. Bergmann and H. Machemer, *Ber.*, **63**, 316 (1930).

(44) F. Müller, *Helv. Chim. Acta*, **22**, 217 (1939).

(45) M. L. Wolfrom and L. W. Georges, *J. Am. Chem. Soc.*, **59**, 282 (1937); M. L. Wolfrom, L. W. Georges and J. C. Sowden, *ibid.*, **60**, 1026 (1938); M. L. Wolfrom and J. C. Sowden, *ibid.*, **60**, 3009 (1938); M. L. Wolfrom, J. C. Sowden and E. N. Lassettre, *ibid.*, **61**, 1072 (1939).

(46) M. Bergmann and H. Machemer, *Ber.*, **63**, 2304 (1930).

(47) E. Schmidt, K. Meinel, W. Jandebeur and W. Simson, *Cellulosechem.*, **13**, 129 (1932); E. Schmidt, R. Schnegg and Margarete Hecker, *Naturwissenschaften*, **21**, 206 (1933); E. Schmidt, Margarete Hecker, W. Jandebeur and M. Atterer, *Ber.*, **67**, 2037 (1934); E. Schmidt, R. Schnegg, W. Jandebeur, Margarete Hecker and W. Simson, *ibid.*, **68**, 542 (1935); E. Schmidt, W. Jandebeur, Margarete Hecker, R. Schnegg and M. Atterer, *ibid.*, **69**, 366 (1936).

(48) E. Heymann and Gertrude Rabinov, *J. Phys. Chem.*, **45**, 1152, 1167 (1941); *Trans. Faraday Soc.*, **38**, 209 (1942).

(49) A. M. Sookne and M. Harris, *J. Research Natl. Bur. Standards*, **25**, 47 (1940); A. M. Sookne, C. H. Fugitt and J. Steinhardt, *Am. Dyestuff Reptr.*, **29**, 333 (1940).

(50) T. Kleinert, G. Hingst and Ingeborg Simmler, *Kolloid-Z.*, **108**, 144 (1944).

primarily to cleavage of these foreign cross linkages and only secondarily to the fission of the anhydro-D-glucose chains. The suggestion that more easily hydrolyzed linkages than those existing between anhydro-D-glucose units are present in the cellulose molecule had been made earlier by Schulz and Husemann.[51] The interpretation placed on the data obtained by these workers was that native cellulose is a homogeneous material with a degree of polymerization of 3,100 anhydro-D-glucose units but contains five equally spaced linkages along the molecule which hydrolyze 1000 times faster than the other normal linkages. Somewhat similar to the concept of Kleinert, Hingst and Simmler is the more recent concept of Pacsu and Hiller,[52] in which cross linkage between anhydro-D-glucose chains is achieved through hemiacetal linkages of the hydrated aldehyde group of such simple sugars as D-glucose, cellobiose and cellotriose with any of the hydroxyl groups along the polymer chain. Of interest in this connection are the observations of Freudenberg and Soff,[53] that methyl α- and β-D-glucosides, upon treatment with an acetolyzing mixture, may be partially converted into heptaacetyl-D-glucose. This same material may be isolated from the acetolysis products of cellulose. The structure of cellulose as given by Pacsu would indicate that a somewhat similar reaction occurs *in vivo*, resulting in the bonding of the long anhydro-D-glucose chains by primary valence linkages.

4. *Mode of Linkage between the Basic Units*

It has been previously noted that positions 1, 4 and 5 in the basic D-glucose unit of cellulosic materials are involved in some mode of chemical linkage. In order to determine the nature of this chemical linkage, degradation products of purified cellulosic materials were investigated.

a. Cellobiose. In 1879, Franchimont reported that the prolonged treatment of cotton fibers with acetic anhydride and sulfuric acid yielded a crystalline material.[54] This was later found to be an octaacetate of a new disaccharide[55] which consisted of two D-glucose residues not fermented by yeast maltase. The ready hydrolysis of the disaccharide by emulsin[56] indicated that the linkage was of the β-D configuration. The methylation of crystalline methyl heptaacetyl-β-cellobioside,[55] or of

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(51) G. V. Schulz and E. Husemann, *Z. physik. Chem.*, **B52**, 23 (1942); G. V. Schulz, *ibid.*, **B52**, 50 (1942).

(52) E. Pacsu and L. A. Hiller, Jr., *Textile Research J.*, **16**, 243 (1946).

(53) K. Freudenberg and K. Soff, *Ber.*, **70**, 264 (1937).

(54) A. P. N. Franchimont, *Ber.*, **12**, 1941 (1879); *Rec. trav. chim.*, **18**, 472 (1899); see G. Zemplén, A. Gerecs and I. Hadacsy, *Ber.*, **69**, 1827 (1936).

(55) Z. H. Skraup and J. König, *Monatsh.*, **22**, 1011 (1901).

(56) E. Fischer and G. Zemplén, *Ann.*, **365**, 1 (1909).

cellobiose,[57] gave crystalline methyl heptamethyl-β-cellobioside, which upon hydrolysis yielded one mole each of 2,3,4,6-tetramethyl-D-glucose and 2,3,6-trimethyl-D-glucose. This result proved that cellobiose was a 4 or 5-β-D-glucopyranosyl-D-glucose.

The first evidence produced, showing that the β-D-glucosyl radical was attached to position 4 of D-glucose, was that of Zemplén,[58] who degraded the oxime of cellobiose to glucosyl-D-arabinose by the method of Wohl.[59] Upon degrading the oxime of glucosyl-D-arabinose, glucosyl-D-erythrose was obtained in poor yield; the latter did not form an osazone. This was taken as evidence that position 2 in the D-erythrose residue, or position 4 in the original D-glucose residue, was blocked by linkage to the glucosyl radical. This result was confirmed shortly by Haworth and coworkers,[60] who oxidized cellobiose to cellobionic acid, which upon methylation yielded methyl octamethylcellobionate. When subjected to acid hydrolysis this product gave a 92% yield of 2,3,4,6-tetramethyl-D-glucose and an 80% yield of crystalline 2,3,5,6-tetramethyl-D-gluconic lactone. These results are regarded as proof that cellobiose is 4-(β-D-glucopyranosyl)-D-glucopyranose. This structure received additional confirmation from the synthesis of octamethylcellobiose by the condensation of 2,3,4,6-tetramethyl-α-D-glucopyranosyl chloride with methyl 2,3,6-trimethyl-β-D-glucopyranoside.[61] Similarly, methyl heptaacetyl-β-cellobioside was synthesized by condensing 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide with methyl 2,3,6-triacetyl-β-D-glucopyranoside.[62] It was left to Freudenberg and Nagai to synthesize cellobiose itself[63] by condensing 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide with 1,6-anhydro-D-glucose. The mixture containing the partially acetylated anhydrocellobiose thus obtained, upon controlled acetolysis gave cellobiose octaacetate in low yield, which upon alkaline hydrolysis yielded cellobiose. The method of Hudson and coworkers,[64] appears to be the best yet devised and involves the condensation of 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide with 2,3-isopropylidene-D-mannosan $< 1,5 > \beta < 1,6 >$. Upon mild hydrolysis, the acetone residue

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(57) W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, **119**, 193 (1921).

(58) G. Zemplén, *Ber.*, **59**, 1254 (1926).

(59) A. Wohl, *Ber.*, **26**, 730 (1893); **30**, 3101 (1897); **32**, 3666 (1899).

(60) W. N. Haworth, C. W. Long and J. H. G. Plant, *J. Chem. Soc.*, 2809 (1927); W. N. Haworth, E. L. Hirst and E. J. Miller, *ibid.*, 2436 (1927).

(61) K. Freudenberg, C. C. Andersen, Y. Go, K. Friedrich and N. W. Richtmyer, *Ber.*, **63**, 1961 (1930).

(62) B. Helferich and H. Bredereck, *Ber.*, **64**, 2411 (1931).

(63) K. Freudenberg and W. Nagai, *Ann.*, **494**, 63 (1932).

(64) W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **63**, 1724 (1941); **64**, 1289 (1942).

was removed and following acetolysis, crystalline 4-β-D-glucopyranosyl-D-mannose octaacetate (epicellobiose octaacetate) was obtained. This product was then converted to heptaacetyl-4-α-D-glucopyranosyl-D-mannosyl bromide, then to cellobial hexaacetate, thence to α- and β-cellobiose octaacetate and finally to cellobiose.

The synthesis of cellobiose octaacetate by the reaction of the sodium derivative of 1,2,3,6-tetraacetyl-D-glucopyranose with 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide has recently been reported.[65] Deacetylation resulted in the formation of cellobiose.

Although the structure of cellobiose may now be considered proven, there still remains the possibility that this substance results from the condensation of D-glucose anhydride molecules liberated by the acetolysis mixture, rather than being a true degradation product.[66] The yield of cellobiose octaacetate of 16–19% recorded by Ost,[67] and of 35–37% obtained by Madsen,[68] and Freudenberg,[68a] is of considerable importance in this connection. Friese and Hess,[69] soon after, obtained the highest yield yet reported from a cellulosic material, namely, 50–51%. Following the procedure employed by Friese and Hess, Spencer[69a] obtained yields of only 37%. However, modification of the procedure to correspond more closely to that employed by Madsen resulted in a yield of 46.5%. The mathematical treatment of the problem by Freudenberg[70] and by Kuhn[71] has shown that theoretically 29.8–32% of cellobiose may be obtained from a pure cellulosic material. On the other hand, if the disaccharide were entirely stable in the acetolysis mixture, then 66.7% should be obtained. It is known, however, that cellobiose octaacetate is not completely removed from the acetolysis mixture by crystallization; thus the yield realized lies between the two extremes. The yield of 50–51% is reasonable and lends strong support to the contention that cellobiose exists preformed as a fundamental molecular constituent of cellulosic materials.

b. Cellotriose, Cellotetraose and Cellopentaose. During the acetolysis of cellulosic materials, oligosaccharide acetates other than cellobiose

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(65) Violet E. Gilbet, F. Smith and M. Stacey, *J. Chem. Soc.*, 622 (1946); see W. N. Haworth, *Proc. Roy. Soc. London*, **186A**, 1 (1946).

(66) K. Hess, W. Weltzien and R. Singer, *Ann.*, **443**, 90 (1925).

(67) H. Ost, *Ann.*, **398**, 323 (1913).

(68) Madsen, Ph. D. Dissertation, Hanover (1917).

(68*a*) K. Freudenberg, *Ber.*, **54**, 771 (1921).

(69) H. Friese and K. Hess, *Ann.*, **456**, 39 (1927).

(69*a*) C. C. Spencer, *Cellulosechem.*, **10**, 61 (1929).

(70) K. Freudenberg, *Ber.*, **54**, 767 (1921).

(71) W. Kuhn, *Ber.*, **63**, 1523 (1930). See H. Mark, "High Polymers," Vol. 2, "Physical Chemistry of High Polymeric Systems," p. 327, Interscience Publishers, Inc., New York (1941).

octaacetate are formed. Following the suggestion of Bertrand and Benoist,[72] cellotriose was obtained in crystalline form by Ost.[73] Further acetolysis of cellotriose yielded cellobiose octaacetate and D-glucose pentaacetate.[74] The acetolysis of trimethylcellulose followed by methylation was found to yield the fully methylated methyl glycosides of D-glucose, cellobiose and cellotriose.[75] Upon hydrolysis, the completely methylated cellotrioside gave 2,3,4,6-tetramethyl-D-glucose and 2,3,6-trimethyl-D-glucose in a ratio of 1:2. Under carefully controlled conditions of acetolysis a product was obtained which, upon simultaneous deacetylation and methylation, yielded methyl heptamethyl-β-cellobioside in addition to methyl tetramethyl-D-glucoside, thus indicating the existence of the cellobiose linkage in cellotriose.

The final proof of structure was furnished by the work of Freudenberg and Nagai,[63] who synthesized methyl decamethyl-β-cellotrioside by condensing methyl 2,3,6-trimethyl-D-glucoside with heptamethyl cellobiosyl chloride. Despite the low yield obtained, this synthesis is generally regarded as confirming the hypothesis that cellotriose is β-cellobiosyl-D-glucose, and that the linkage occupies position 4 in the reducing (D-glucose) unit.

The observation that concentrated hydrochloric acid (41 to 42%) would dissolve cellulosic materials at room temperature led to the development of a second method for obtaining homogeneous degradation products.[74,76] In addition to D-glucose, cellobiose and cellotriose identical with those obtained by acetolysis, two new oligosaccharides were isolated in crystalline form, namely, cellotetraose and cellopentaose. The structure of cellotetraose was shown by hydrolysis following methylation. Thus, methyl tridecamethylcellotetraoside,[77] upon hydrolysis gave a final specific rotation close to that calculated for one molecular equivalent of 2,3,4,6-tetramethyl-D-glucose and three molecular equivalents of 2,3,6-trimethyl-D-glucose. In the case of cellopentaose, the evidence is not so clear cut; in fact, this sugar was first thought to be cellohexaose, but later work on the viscosity-molecular weight relationship,[78] indicated this to be incorrect. Molecular weight determinations

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(72) G. Bertrand and S. Benoist, *Compt. rend.*, **176,** 1583 (1923); **177,** 85 (1923); *Bull. soc. chim.*, [4] **33,** 1451 (1923); [4] **35,** 58 (1924).

(73) H. Ost, *Z. angew. Chem.* **39,** 1117 (1926); J. C. Irvine and G. J. Robertson, *J. Chem. Soc.*, **128,** 1488 (1926); W. N. Haworth, E. L. Hirst and O. Ant-Wuorinen, *ibid.*, 2368 (1932); K. Hess and K. Dziengel, *Ber.*, **68,** 1594 (1935).

(74) L. Zechmeister and G. Tóth, *Ber.*, **64,** 854 (1931).

(75) W. N. Haworth, E. L. Hirst and H. A. Thomas, *J. Chem. Soc.*, 824 (1931).

(76) R. Willstätter and L. Zechmeister, *Ber.*, **46,** 2401 (1913).

(77) K. Freudenberg, K. Friedrich and Ilse Bumann, *Ann.*, **494,** 41 (1932).

(78) H. Staudinger and E. O. Leupold, *Ber.*, **67.** 479 (1934).

by the Rast method, which had given the correct values for fully acetylated D-glucose, cellobiose, cellotriose and cellotetraose, also indicated that this acetylated sugar was a pentaose. In studying the series of oligosaccharides isolated, further evidence was found that the chemical behavior of this sugar was similar to that expected for a pentaose. Thus, cellobiose, cellotriose, cellotetraose and cellopentaose showed the property of mutarotation in aqueous solution and of copper reduction. Acetylation with acetic anhydride in the presence of pyridine yielded crystalline acetates, except in the case of cellopentaose, and upon deacetylation yielded the original sugar. In addition to the Rast method for determining the molecular weight, titration of the reducing groups with alkaline hypoiodite and analysis of the crystalline phenylosazones gave results which agreed with the structures already indicated.

Dickey and Wolfrom[78a] separated a cellulose acetolyzate into a polymer-homologous series of crystalline sugar acetates complete from α-D-glucose pentaacetate through α-cellohexaose eicosaacetate by the application of chromatographic procedures.

In contrast with the linkage present in cellobiose it will be noted that in the case of the higher oligosaccharides more than one type of linkage between units may be thought of as being present, the number of types of linkages reaching three in cellotetraose. This is readily evident from the schematic drawing shown in Fig. 1.

Upon acid hydrolysis of these materials it would be expected that each type of D-glucosidic bond would undergo cleavage at a different rate. Two methods were used by Freudenberg[79] and by Kuhn[80] for determining the value of the hydrolysis constants of each of the isolated oligosaccharides, namely, (a) change in optical rotation with time, and (b) change in the number of reducing groups with time, by titration with alkaline hypoiodite. Considering the hydrolysis of the oligosaccharides as being a first-order reaction, these workers determined the values of the initial reaction-rate constants by extrapolating to zero time. In the case of cellobiose the rate of hydrolysis was a constant, $K_2 = 1.07 \times 10^{-4}$, throughout the course of the reaction, but with cellotriose the rate deviated from constancy, $K_3 = 0.636 \times 10^{-4}$, as the reaction progressed. From a consideration of the structure of cellotriose, it will be noted that regardless of the type of linkage which undergoes cleavage, cellobiose and D-glucose are produced. Cellobiose, thus liberated, undergoes hydrolysis at a definite known rate, K_2. Inasmuch as it is impossible to determine

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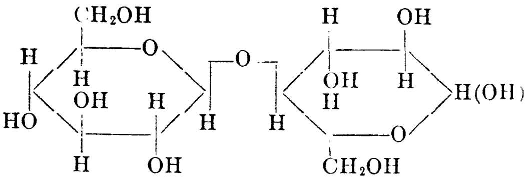
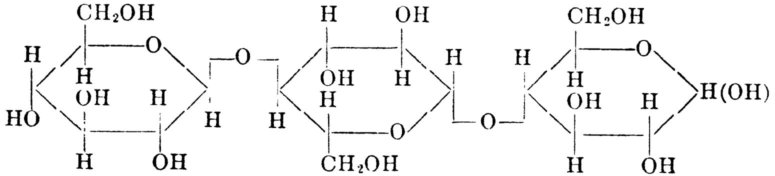
(78a) E. E. Dickey and M. L. Wolfrom, *J. Am. Chem. Soc.*, *in press* (1948).

(79) K. Freudenberg, "Tannin, Cellulose, Lignin," pp. 97–106, J. Springer, Berlin (1938).

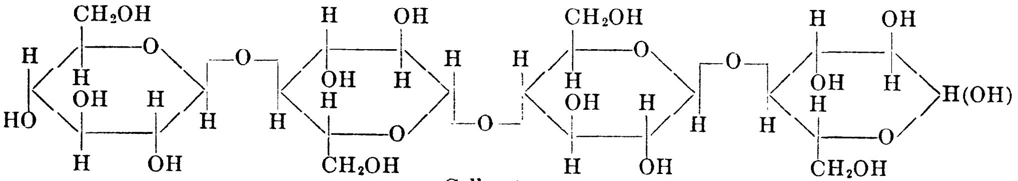
(80) W. Kuhn, *Ber.*, **63**, 1503 (1930).



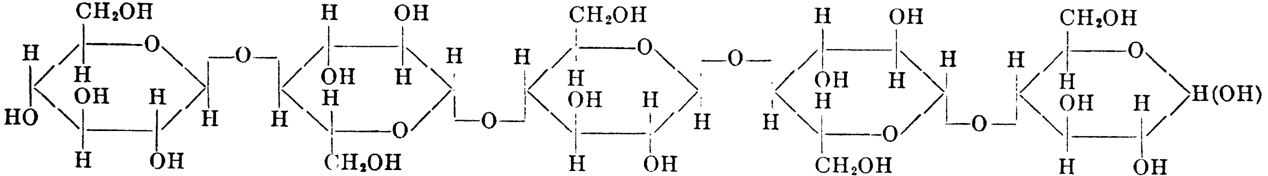
Cellobiose



Cellotriose



Cellotetraose



Cellopentaose

FIG. 1.—Structures of oligosaccharides derived from cellulose.

which of the two linkages in cellotriose is hydrolyzed at the faster rate, it was recognized that the initial rate constant, K_3, perhaps represented an average of the two individual rate constants. However, using the average constants, K_2 and K_3, already obtained, the degree of hydrolysis, α, at any time, t, could be predicted making use of equation 1.[81] The

$$1 - \alpha = \left(\frac{K_2 - K_3}{K_2 - 2K_3}\right) e^{-2K_3t} - \left(\frac{K_3}{K_2 - 2K_3}\right) e^{-K_2t} \qquad (1)$$

results agreed well with the determined values.

The presence of three types of glycosidic linkages in cellotetraose renders the analysis of the hydrolysis rate more difficult than in the case of cellotriose. If the initial cleavage occurs between units 2 and 3, two molecules of cellobiose would be formed at the rate K_m, which in turn would hydrolyze at the constant rate, K_2. On the other hand, if the cleavage occurs between units 1 and 2, or 3 and 4, cellotriose and D-glucose would be formed at the average rate, K_3. The cellotriose produced would then hydrolyze at the average rate, K_3, as already considered. The ratio, q, of K_3 to K_m is then the controlling factor in the hydrolysis of cellotetraose. Since all the factors, q, K_2, K_3, K_4, and the time, t, are known except the ratio, q, it was shown by Kuhn,[80] that the value of q could be calculated. It was thus possible to predict the degree of hydrolysis, α, at any time, t, by the use of an exponential expression for cellotetraose similar to that for cellotriose, the predicted values being in good agreement with experimentally determined values. Further consideration of the problem,[82] led to calculated hydrolysis constants in almost exact agreement with the experimentally determined constant, $K_4 = 0.506$ to 0.520×10^{-4}. The similarity in magnitude of the rate constants K_2, K_3 and K_4 to the rate constants characteristic of β-D-glucopyranosides has been generally accepted as kinetic evidence that the linkages between the basic units of structure in cellobiose, cellotriose and cellotetraose are similar and of this kind. Similar studies on cellopentaose have not been reported. The extension of these hydrolysis studies to purified cotton fiber has shown that the types of glycosidic linkages present are similar to those present in cellotetraose, although some assumptions had to be made in treating the problem mathematically.

Additional proof that only β-D-glucopyranoside linkages are present in the isolated oligosaccharides is furnished by the molecular rotation of

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(81) W. Kuhn, Carolina Molster and K. Freudenberg, *Ber.*, **65**, 1179 (1932).

(82) F. Klages, *Ber.*, **65**, 302 (1932); *Z. physik. Chem.*, **A159**, 357 (1932); K. Freudenberg and W. Kuhn, *Ber.*, **65**, 484 (1932); W. Kuhn, *Z. physik. Chem.*, **A159**, 368 (1932).

these materials and their derivatives.[77,83] The assumption was made that the molecular rotation of the oligosaccharides and of cellulose, was an additive function of the optical rotations contributed by its constituent units. As may be seen from the schematic representations of cellobiose, cellotriose, cellotetraose and cellopentaose, three types of units are present depending upon the relative position of the basic unit, namely: the reducing end group, a; the non-reducing end group, e; and the intermediate group, m. In cellobiose the intermediate group, m, is absent, but in cellotriose, one of each of the types of units is present. As the chain length is increased the value of m becomes equal to $n - 2$, in which n is the total number of units present. The molecular rotation $[M]_n$ of the oligosaccharides with a total of n units can then be expressed by the summarization,

$$[M]_n = a + e + (n - 2)m; \tag{2}$$

then for each average unit of structure,

$$\frac{[M]_n}{n} = \frac{a + e + (n - 2)m}{n} = a + e - m + \frac{n - 1}{n}(2m - a - e). \tag{3}$$

If the type of linkage is entirely uniform, a linear relation should be found when $\frac{[M]_n}{n}$ is plotted against $\frac{n-1}{n}\cdot$ In studying the series cellobiose, cellotriose, cellotetraose, cellopentaose and cellulose before and after acetylation or methylation, the observed $[M]_n/n$ values, in an appropriate solvent, were found to fall on a straight line.

c. Cellodextrins. Controlled acetolysis of cellulosic materials results in the formation of cellodextrin acetate,[84] which upon alkaline hydrolysis yields cellodextrins. This product was first thought to be chemically homogeneous and the name "biosan" was assigned to it because it was thought to be composed basically of two anhydro-D-glucose units.[85]

The cellodextrins obtained in this manner, or by the prolonged action of concentrated mineral acids on cellulosic materials,[86] are characterized by their high reducing power. Upon further controlled acid hydrolysis or acetolysis, a series of homogeneous oligosaccharides may be obtained from the cellodextrins identical with those obtained from the original cellulosic materials. It is thus evident that the cellodextrins are heterogeneous degradation products intermediate between the original cellulo-

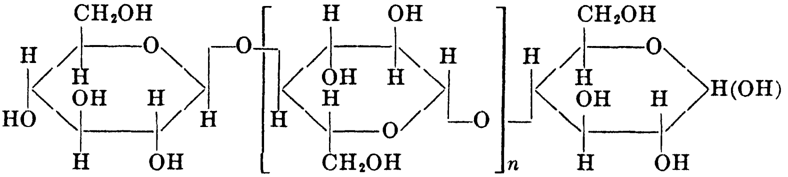
(83) K. Freudenberg and G. Blomqvist, *Ber.*, **68**, 2070 (1935).

(84) K. Hess and W. Weltzien, *Ann.*, **435**, 46, 58 (1923).

(85) K. Hess and H. Friese, *Ann.*, **450**, 40 (1926); K. Hess, *Ber.*, **63**, 518 (1930).

(86) M. Hönig and S. Schubert, *Monatsh.*, **7**, 455 (1886); H. Ost, *Z. angew. Chem.*, **25**, 1996 (1912); M. Bergmann and E. Knehe, *Ann.*, **445**, 1 (1925).

sic material and the homogeneous isolated oligosaccharides.[87] Hydrolysis of the methylated cellodextrins gave 2,3,4,6-tetramethyl-D-glucose and 2,3,6-trimethyl-D-glucose in varying average ratios[88] ranging from 1:10 to 1:30. The number of recurring anhydro-D-glucose units in the average cellodextrin molecule was thus in the range of 10 to 30. Confirmation of this result was reported by Staudinger and coworkers[89] from viscosity measurements on these heterogeneous oligosaccharides. The structural formula of the cellodextrins may thus be written as follows.



Cellodextrin

In this, n is an integer representing an average number of D-glucose anhydride units. The linkages between the units are of the β-D-configuration by analogy with the homogeneous oligosaccharides previously considered. The order of magnitude of the hydrolysis rate constants as well as the molecular rotation of the cellodextrins and derivatives indicate that the types of linkages between the basic units of structure are the same and similar to those present in the homogeneous isolated oligosaccharides.[90]

d. Cellulose. The inference may now be drawn that the mode of linkage between the basic units of structure in the anhydro-D-glucose polymer component of cellulosic materials is the same as that in the hydrolytic cleavage products. The validity of this inference has been shown in the case of degraded cellulosic materials but the same cannot be claimed for native cellulose since neither the presence of end groups, nor the absence of cross linkages, has been definitely established.[34,35,36,39,50,51,52] However, the majority of the linkages must be the same as those found in the isolated homogeneous oligosaccharides, cellotetraose and the cellodextrins. Thus, the homogeneous acid hydrolysis of purified cotton fiber when followed by optical rotation and by hypoiodite titrations gave an initial rate constant, $K_n = 0.305 \times 10^{-4}$, which was of the same order of magnitude as similarly obtained values described above for cellobiose, cellotriose, and cellotetraose.[82] If the simplifying assumption is made

(87) K. Freudenberg, E. Bruch and Helene Rau, *Ber.*, **62**, 3078 (1929).

(88) W. N. Haworth and H. Machemer, *J. Chem. Soc.*, 2372 (1932).

(89) H. Staudinger and H. Freudenberger, *Ber.*, **66**, 76 (1933).

(90) K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz and G. Steinbrunn, *Ber.*, **63**, 1510 (1930).

that the linkages adjacent to the reducing end of the cellulose molecule are cleaved at the rate, K_2, similar to that of cellobiose, and all other linkages are cleaved at the rate, K_n, it was shown by Klages and by Kuhn,[82] that the unhydrolyzed residue, $1 - \alpha$, at any time, t, may be calculated from an exponential function involving K_2, K_n and t. A more valid treatment of the problem in which the assumption is made that the linkages adjacent to both end groups of the cellulose molecule were cleaved at the rate K_3, similar to that of cellotriose, and all other linkages were cleaved at the rate K_n, gave an exponential function whose calculated $1 - \alpha$ values agreed even more closely with the experimentally determined values. Further studies[91] have shown that the hydrolysis rate constant, K_n, is independent of the number of basic units present in the average cellulose molecule in the range of 130 to 1500. These results indicate that at least in partially degraded cellulosic materials no linkages are present in a detectable amount which are more easily cleaved than the β-D-glucoside linkage, but the presence of linkages more difficult to cleave is not excluded. These conclusions are refuted by the recent work of Hiller and Pacsu,[92] who claim that weak acids do not hydrolyze the 1,4-glycosidic linkages to an appreciable extent, but rather hydrolyze hemiacetal linkages which connect the terminal open-chain D-glucose units of the primary anhydro-D-glucose chain molecules through hemiacetal linkages involving open-chain molecules of simple sugars, giving rise to a three dimensional network of secondary chain molecules. These hemiacetal linkages are present to the extent of about one for every 548 anhydro-D-glucose units and are hydrolyzed about 1000 times faster than the normal 1,4-D-glucosidic linkages. Upon extending these investigations, Pacsu[92a] has now concluded that the building units of the cellulose fiber, "*limit hydrocellulose*," which is devoid of acid-sensitive linkages, has the probable degree of polymerization value of $2^8 = 256$, or approximately one-half that previously designated. The reservation is further made that should electron microscopic investigations reveal that the length of a crystallite is 660 Å., as present x-ray studies would seem to indicate, then the *D.P.* (degree of polymerization) value would become $2^7 = 128$.

As previously considered in the case of the oligosaccharides, the molecular rotation of cellulose is an additive function of the optical rotations contributed by the constituent basic units.[77,83] When the number of intermediate basic units become infinitely large, the m value in equation 3 above, may be expressed as $\frac{[m]_\infty}{\infty}$, since the contribution of the optical

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(91) G. V. Schulz and H. J. Löhmann, *J. prakt. Chem.*, [2] **157**, 238 (1941).

(92) L. A. Hiller, Jr., and E. Pacsu, *Textile Research J.*, **16**, 490, 564 (1946).

(92*a*) E. Pacsu, *Textile Research J.*, **17**, 405 (1947).

rotations of the end groups becomes negligible. Equation 3, on rearrangement, may now be written as follows.

$$\frac{[M]_n}{n} = \frac{a+e}{n} + \frac{n-2}{n}\left(\frac{m_\infty}{\infty}\right) \tag{4}$$

The observed molecular rotations of cellobiose, $\frac{a+e}{2}$, and of cellulose, $\left(\frac{m_\infty}{\infty}\right)$, when replaced in equation 4 by their equivalent designations, $\left[\frac{M_2}{2}\right]$ and $\left[\frac{M_\infty}{\infty}\right]$, respectively, give the following general expression.

$$\frac{[M]_n}{n} = \frac{2}{n}\left(\frac{[M]_2}{2}\right) + \frac{n-2}{n}\left(\frac{[M]_\infty}{\infty}\right) \tag{5}$$

The molecular rotations of the homogeneous oligosaccharides may now be calculated from the observed molecular rotations of cellulose and cellobiose, or conversely, the molecular rotation of cellulose may be calculated if the molecular rotations of the homogeneous oligosaccharides are known. The agreement between the observed molecular rotations and the calculated values for cellulose and the oligosaccharides, as well as for the completely acetylated and methylated derivatives of each, has, with few exceptions, been found to be very satisfactory. It is claimed by Freudenberg that a difference between calculated and observed molecular rotations large enough to be easily detected would exist if there were as few as one α-D-linkage relative to 100 β-D-linkages in the cellulose molecule. This agreement between calculated and observed molecular rotations has been generally accepted as proof that the assumed uniformity of linkages is actually present in cellulose and the oligosaccharides.

From the evidence presented, the structural formula of native cellulose may be schematically represented as in Fig. 2, neglecting for the moment the concepts of cross linking, and the fact that by common usage of the term, a cellulosic fiber material containing no detectable, or many, end groups is still called cellulose.

While it is not known that the end groups of the true cellulose molecule are chemically linked to form a cyclic molecular structure, as indicated, the chemical behavior of undegraded, purified cellulosic materials suggests this possibility.[36,93] In the case of the cellodextrins, the value of n varies, but probably reaches a maximum of 50 anhydro-D-glucose units; on the other hand, the value of n in cellulose is obviously larger than 50. This problem will be considered later in greater detail.

(93) K. Hess and E. Steurer, *Ber.*, **73**, 669 (1940); K. H. Meyer, *ibid.* **70**, 266 (1937).

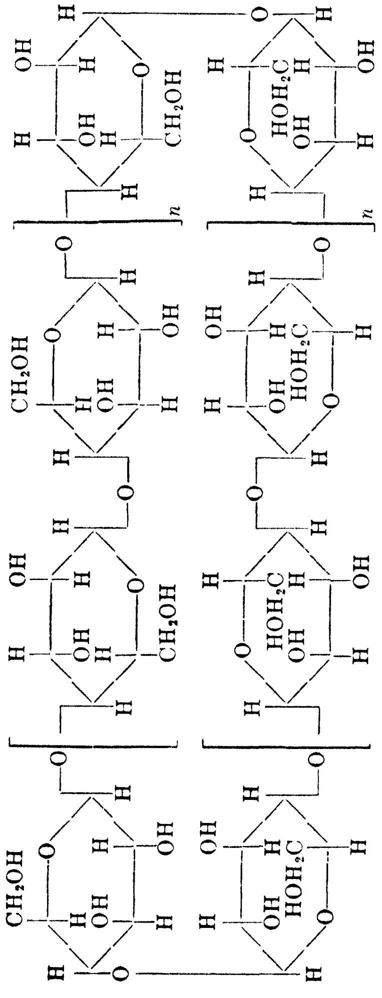

FIG. 2.—Native cellulose according to the cyclic concept.

IV. Degree of Polymerization of Cellulose

It has long been known that subjection of cellulosic fiber materials to strenuous mechanical or chemical treatments results in a weakened or degraded condition. As previously indicated in the case of the oligosaccharides, the reducing power of these materials increase as the number of anhydro-D-glucose units decrease. Similarly, as the number of anhydro-D-glucose units increase, such physical properties of the oligosaccharides and their derivatives as solubility, melting and boiling point, molecular rotation, dispersion and viscosity approach that of the recognized cellulose component of cellulosic materials. When the number of basic units is near 30, dispersions of the oligosaccharide in solvents begin to show the colloidal properties characteristic of cellulose. Thus, a concept has evolved which relates the homogeneous oligosaccharides to the heterogeneous oligosaccharides or cellodextrins, and these in turn, to degraded cellulose and finally cellulose itself. According to this concept, the variation in chemical and physical properties of this series of materials is attributed to the average number of anhydro-D-glucose units present in each. This process of reasoning whereby the simple is used to explain the complex is of course valid only to the extent that the former is truly representative of the latter. Based on the assumption that the molecular structure of the simple oligosaccharides adequately represents the type of molecular structure present in complex cellulosic materials, methods have been developed for estimating the average number of anhydro-D-glucose units present in both degraded and native cellulose molecules. The average degree of polymerization (*D.P.*) of cellulosic material may be expressed as the average molecular weight by multiplying the former by the molecular weight of the anhydro-D-glucose unit (162). The methods developed for determining the degree of polymerization or chain length of cellulosic materials may be discussed under two headings: (1) chemical methods and (2) physical methods.

1. *Chemical Methods*

It will be observed that the structural formula of cellodextrin (page 201) is of the open-chain type. This formula is also representative of the generally accepted formula of degraded or modified true cellulose. Since each degraded cellulose molecule is characterized by the presence of end groups which upon treatment with various reagents, react differently than the remainder of the basic units, a quantitative determination of either of the end groups affords a means of determining the total number of basic units present in the average molecule. It is thus apparent that a small percentage of either end group indicates a long average chain length, whereas a large percentage indicates a short average chain length, as in the case of the oligosaccharides. The number of end groups relative to the total (average) number of basic units may be calculated from a determination of potential reducing groups or non-reducing end groups. From these data the average degree of polymerization or chain length is determined.

a. Reducing End Groups. When the various reactions indicative of the presence of reducing groups in cellulosic fiber materials are used for chain-length determinations, it must be assumed that every chain molecule terminates in a hemiacetal group and that the reducing power is due solely to these preformed, potentially aldehydic terminal groups. The validity of these assumptions is open to question, as will be shown later, but in some of the methods developed, these objections have largely been overcome. The most outstanding method in this regard is a modification[94] of the alkaline hypoiodite method of Bergmann and Machemer.[46] In this modification a correction is determined and applied for the side reaction involving the consumption of iodine,[34] even under the mild conditions of 0°C. and a *p*H of 10.6. In this method the aldehyde group is oxidized to a carboxyl group and the results of an alkaline hypoiodite determination may be verified by titrating the carboxyl groups thus produced with *o*-nitrophenolate. Despite the added precautions the method has been criticized and further modification recommended.[95] The aldehyde content of purified cotton, when determined in this manner, was found to be zero, but a series of degraded cotton samples (hydrocellulose) gave calculated *D.P.* values varying from 402 to 1166.

The use of dilute permanganate in sulfuric acid at 0°C. as a selective oxidant for the estimation of reducing end groups in cellulosic materials has recently been suggested.[96] Employing this procedure it was found that surgical cotton had a calculated *D.P.* of 290; cotton linters pulp, 1650; and viscose rayon, 165.

The method of Wolfrom and coworkers[45] is unique in that use is made of the reaction of aldehydic end groups of hydrocellulose and certain of its derivatives with ethyl mercaptan in the presence of fuming hydrochloric acid. The extent of mercaptal formation is determined by the rate at which reducing groups are liberated by hydrolysis. It is then necessary to extrapolate to zero time to obtain an estimate of the *D.P.* value of the starting material. Methylcellulose obtained by the methyla-

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(94) A. R. Martin, L. Smith, R. L. Whistler and M. Harris, *J. Research Natl. Bur. Standards*, **27**, 449 (1941); *Am. Dyestuff Reptr.*, **30**, 628 (1941); H. A. Rutherford, F. W. Minor, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **29**, 131 (1942).

(95) E. Pacsu, *Textile Research J.*, **16**, 105 (1946).

(96) L. A. Hiller, Jr., and E. Pacsu, *Textile Research J.*, **16**, 318 (1946).

tion of a secondary cellulose acetate gave an average extrapolated *D.P.* value of 400. No values have been published on the *D.P.* of native cellulose determined in this manner.

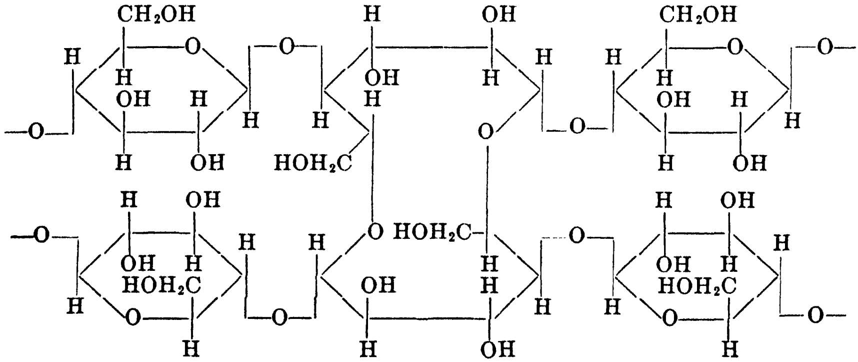
Less reliable reducing end group methods include the "copper number" of Schwalbe,[40] the "silver number" of Götze[41] and methods employing phenylhydrazine.[42,43,44] The *D.P.* values obtained by these methods, however, are usually of about the same order of magnitude as those obtained by the method of Bergmann and Machemer.[46]

b. Non-reducing End Groups. Attention has previously been called to the presence of tetramethyl-D-glucose among the hydrolysis products of degraded cellulosic material regenerated from a secondary cellulose acetate and methylated with dimethyl sulfate in the presence of alkali and oxygen.[33] From the yield of tetramethyl-D-glucose it was concluded that the cellulosic material had an average chain length of 100 to 200 anhydro-D-glucose units. Exclusion of oxygen during the methylation of purified fibrous cellulosic material and a variation in the fractionation technique led Hess and coworkers[34] to the result previously stated, namely, that no tetramethyl-D-glucose could be isolated from the hydrolyzate of the methylated product. It was therefore concluded that there was either an infinite number of anhydro-D-glucose units in the undegraded cellulose molecule or that cross linkages involving the end groups existed between cellulose molecules. In later experiments, however, traces of tetramethyl-D-glucose were found and the minimum degree of polymerization of the cellulose was calculated to be 10,000.

The conclusion of Hess was soon verified by Haworth and coworkers,[35] in that the methylation of undegraded cotton in a nitrogen atmosphere resulted in the absence of tetramethyl-D-glucose in the hydrolyzate. Nevertheless, they report that cotton slivers, after 15 methylations in a nitrogen atmosphere, yielded tetramethyl-D-glucose corresponding to a chain length of 700 anhydro-D-glucose units. It is quite likely that all traces of oxygen were not eliminated during this drastic methylation since viscosity and osmotic pressure measurements indicated a decrease in average chain length.

To explain the behavior of cellulose when methylated in the presence and absence of oxygen, Haworth assumes that the end groups liberated during methylation in a nitrogen atmosphere combine to form cross linkages between chains, whereas in the presence of air, combination is inhibited. The perfect alignment of the chains to allow this head-to-tail combination of the cellulose molecules, is ascribed to the chains running in opposite directions and being held together by some primary valence cross linkages at various intervals along their length. Association of the chains side by side in this manner offers an explanation for the failure to isolate tetramethyl-D-glucose from a short chain length (*D.P.* 190) methylcellulose as determined by viscosity measurements. The nature of these cross linkages has not been stated as yet but they are thought to be preexistent in cellulosic materials and to occur at intervals of 25 to 30 D-glucose units. If this is the case, then the conclusions drawn from the careful work on the rate of acid hydrolysis and molecular rotation of the oligosaccharides, cellodextrins and cellulose must be considered inadequate.

To explain the discrepancies between the degree of polymerization of cellulose calculated from the determination of tetramethyl-D-glucose in the hydrolyzate of fully methylated cellulose and from viscosity measurements, Hess and coworkers[34] have offered the suggestion that individual anhydro-D-glucose chains are laterally connected by cross linkages. This explanation is similar to that of Haworth's but differs in that the cross linkages do not exist necessarily only between two chains but rather many chains may be involved in a three dimensional structure. This chemical intermeshing of chains would persist in dispersions of cellulose and the degree of polymerization of cellulose calculated from viscosity data would be fictitious. The type of molecular structure in which two kinds of linkages are present has been suggested as a possibility and is rather incompletely shown as follows.



The concept of primary valence cross linking of the anhydro-D-glucose polymer component of cellulosic materials through the functional groups of another molecule or those of groups of molecules has previously been mentioned.[50,52] According to the Pacsu[52] concept, the structure of one type of cellulose could be represented as shown in Fig. 3. Here, the hydrated, open-chain form of cellobiose, in conjunction with the terminal open-chain D-glucose polymer units, holds the long anhydro-D-glucose polymer chains together through hemiacetal linkages. Other simple sugars or sugar derivatives may replace cellobiose in this scheme.

The possibility of the presence of cross linkages in the molecular structure of cellulose has been indicated by the work of Heuser,[97] in cellulose nitrate by the work of McBain and Scott,[98] and, by inference,[99] in cellulose acetate. It is evident that the conclusions which have been drawn from studies of the reducing and non-reducing end groups in cellulosic materials have shaken the foundation of provincial cellulose chemistry.

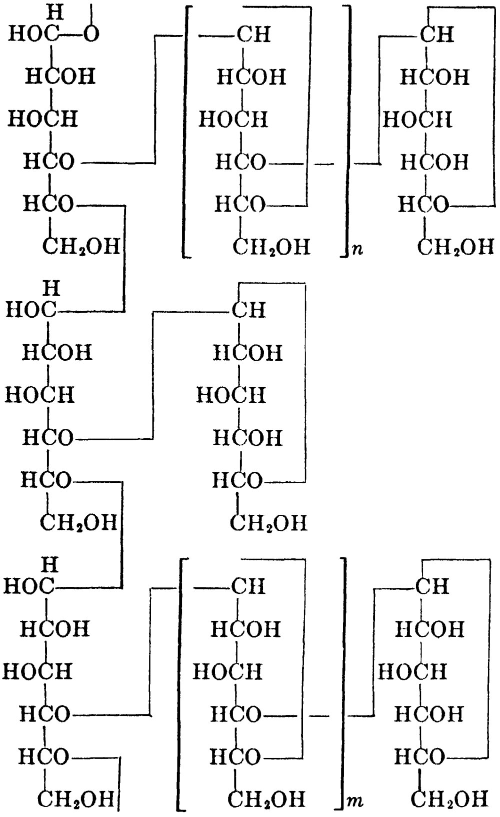


FIG. 3.—Cellulose according to the Pacsu concept.

c. Reducing and Non-reducing End Groups. The complexity of the structure of cellulosic materials is such that any new method of determining end groups may give information of fundamental importance. A

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(97) E. Heuser, *Paper Trade J.*, **122,** 43 (Jan. 17, 1946); E. Heuser, Abstracts of Papers, 110th Meeting of the American Chemical Society, Chicago, Ill., p. 3C (September, 1946).

(98) J. W. McBain and D. A. Scott, *Ind. Eng. Chem.*, **28,** 470 (1936).

(99) P. J. Flory, *J. Am. Chem. Soc.*, **67,** 2048 (1945).

method having such possibilities has recently been published.[100] It depends on the quantitative liberation of formic acid from the reducing and non-reducing end groups of cellulosic materials, using potassium periodate as the oxidizing agent. The reducing end groups yield two moles of formic acid, whereas the non-reducing end groups yield one mole. In this manner it has been shown that the average degree of polymerization of cellulose is at least 1000. The use of this method in conjunction with other end group methods should lead to a better understanding of the types of cross linkages, which may be present in the molecular structure of cellulose.

d. Carboxyl Groups. It has previously been pointed out that when an oxidant was used in the quantitative determination of aldehydic groups in degraded cellulosic materials, the carboxyl groups formed could be estimated independently.[47] The agreement between the carboxyl end group values and the original aldehydic end group values has led to considerable reliance being placed in the carboxyl end group method. When this method, or other carboxyl end group methods, is applied to native purified cellulosic materials for determining the degree of polymerization, the assumption is made that the end groups are carboxyls and that reducing end groups do not exist. These carboxyl groups may be quantitatively determined by adding a known quantity of dilute caustic solution to electrodialyzed cotton and back titrating with dilute acid.[101] In this manner purified native cellulosic material was found to have a carboxyl content of 0.03 to 0.04%. However, Schmidt and coworkers,[47] using a conductometric titration procedure, found 0.28% carboxyl, corresponding to a degree of polymerization of 96. This result could not be reproduced by Neale and Stringfellow,[102] who contend that titration in the presence of sodium chloride is essential for accurate results. Using this modified conductometric titration procedure, standard cotton cloth was found to have a carboxyl content of 0.009% or a *D.P.* of about 3,000, which is a value in good agreement with Staudinger viscosity data.

Recently, Hiller and Pacsu[103] have shown that accurate results may be obtained in the absence of sodium chloride by a direct alkali titration of the carboxyl groups in purified cotton. The carboxyl group content found was 0.02%, but after oxidation with potassium permanganate solution, 0.126% was found. From these data it may be seen that both carboxyl and aldehydic groups are present in the original cellulosic

(100) F. Brown, S. Dunstan, T. G. Halsall, E. L. Hirst and J. K. N. Jones, *Nature*, **156**, 785 (1945).

(101) K. G. Schwalbe and E. Becker, *Ber.*, **54**, 545 (1921); P. Karrer and T. Lieser, *Cellulosechem.*, **7**, 1 (1926); E. Heuser and F. Stöckigt, *ibid.*, **3**, 61 (1922).

(102) S. M. Neale and W. A. Stringfellow, *Trans. Faraday Soc.*, **33**, 881 (1937).

(103) L. A. Hiller, Jr., and E. Pacsu, *Textile Research J.*, **16**, 390 (1946).

material. The degree of polymerization calculated from the total carboxyl group content was 220.

The addition of excess calcium acetate to purified cellulosic material suspended in water, followed by titration of the liberated acetic acid, was suggested by Lüdtke.[104] From the 0.1% carboxyl content obtained, a *D.P.* value of 277 was calculated for the cellulosic material employed. This method has been studied further and comparative data with the earlier conductometric titration procedure have been presented by Heymann and Rabinov,[48] by Kenyon and coworkers,[105] and by Sookne and Harris.[49] The latter workers report that electrodialyzed depectinized cotton has a carboxyl content of 0.045% or a *D.P.* of 616. An iodometric titration procedure for estimating carboxyl groups in cellulosic materials has also been described by Lüdtke.[104] The procedure involves a thiosulfate titration of the iodine liberated from a mixture of potassium iodate and potassium iodide by the carboxyl groups of the cellulosic material. The carboxyl content determined by this method agreed well with that obtained by the calcium acetate method.

The evolution of carbon dioxide by the action of 12% hydrochloric acid on purified cotton fiber to the extent of 0.026 to 0.056%[101,106] has led to the conclusion that carboxyl groups are fundamental constituents of the cellulose molecule. From these values the average chain length of the cellulose molecule can be calculated to be 496 to 1068 anhydro-D-glucose units. These values are essentially in agreement with the results obtained by direct titration of the carboxyl groups with dilute caustic[101] and by various base-combining procedures.[49,105]

The affinity of variously oxidized cellulosic materials for certain basic dyes, notably methylene blue, has long been known.[19] Kenyon and coworkers[105] have recently shown that in the case of nitrogen dioxide oxycelluloses, the methylene blue absorption is a function of the carboxyl group content. Application of the reversible methylene blue method to the determination of carboxyl groups in purified cellulose has been made by Davidson[106a] and by Weber,[107] who was able to show good agreement with the cation-exchange methods previously considered as well as with viscosity and osmotic pressure measurements.

(104) M. Lüdtke, *Biochem. Z.*, **268**, 372 (1934); **285**, 78 (1936); *Angew. Chem.*, **48**, 650 (1935).

(105) E. C. Yackel and W. O. Kenyon, *J. Am. Chem. Soc.*, **64**, 121 (1942); P. A. McGee, W. F. Fowler, Jr., and W. O. Kenyon, *ibid.*, **69**, 347 (1947); C. C. Unruh, P. A. McGee, W. J. Fowler, Jr., and W. O. Kenyon, *ibid.*, **69**, 349 (1947).

(106) R. L. Whistler, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **24**, 13 (1940); R. F. Nickerson and C. B. Leape, *Ind. Eng. Chem.*, **33**, 83 (1941).

(106a) G. F. Davidson, *Shirley Inst. Mem.*, **21**, 47, 85 (1947).

(107) O. H. Weber, *J. prakt. Chem.*, [2] **158**, 33 (1941); E. Husemann and O. H. Weber, *ibid.*, [2] **159**, 334 (1942); *ibid.*, [2] **161**, 1 (1942).

It is thus apparent that there is considerable evidence pointing to the presence in native purified cellulosic materials of acidic groups which must be considered an integral part of the basic structure. Any proposed molecular structure of cellulose which fails to account for the presence of carboxyl groups must therefore be regarded as inadequate.

2. *Physical Methods*

The physical methods for determining the calculated degree of polymerization of cellulosic materials, in contrast to most of the chemical methods, require the disruption of the organized fiber structure. Since no true solvent has been discovered for cellulosic materials, recourse is had to the initial formation of a derivative which renders it solvent dispersible. The quest for a number of years has been for methods of preparing derivatives of cellulosic materials and finding solvents which cause the least change in the fundamental molecular units of structure. In the case of such well-known dispersing agents as cuprammonium hydroxide and the quaternary ammonium bases the fact that complex compound formation precedes dispersion is generally recognized.[108] Inasmuch as there is no absolute criterion for determining whether a cellulosic derivative dispersed in a solvent exists in a "true molecular" state representative of that present in the native organized fiber, physical methods have been the choice ground for theorists. It is now generally recognized that osmotic pressure and ultracentrifugal measurements give a particle size (of the dispersed phase) which is equivalent to the molecular weight only when no aggregation of molecules exists. The degree of polymerization of cellulosic materials determined from viscosity measurements can be no more reliable than the standardization method on which it is based. The standardization method currently in favor is osmotic pressure measurements or ultracentrifugal measurements, although for a time, classical molecular weight measurements on very low members of the polymeric cellodextrins series were used.[109] The possibility that aggregates of cellulose molecules are present even in very dilute dispersions of cellulosic materials cannot be ignored, although there is considerable evidence that the particles are true single chain molecules.[110]

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(108) K. Hess and E. Messmer, *Kolloid-Z.*, **36**, 260 (1925); J. Compton, *J. Am. Chem. Soc.*, **60**, 1807 (1938); W. A. Sisson and W. R. Saner, *J. Phys. Chem.*, **43**, 687 (1939).

(109) H. Staudinger, "Die hochmolekularen organischen Verbindungen," J. Springer, Berlin (1932).

(110) H. M. Spurlin *in* "Cellulose and Cellulose Derivatives," E. Ott, Editor, p. 855, Interscience Publishers, New York (1943).

a. Osmotic Pressure Measurements. Through the pioneering work of Duclaux[111] and of Ostwald[112] it was shown that derivatives of cellulosic materials give measurable osmotic pressures apparently in accordance with the van't Hoff equation,

$$P = \frac{CRT}{M} \tag{6}$$

in which P is the osmotic pressure; C, the concentration; M, the molecular weight; R, the gas constant; and T, the absolute temperature. It was soon recognized that because of association at higher concentration, it was necessary to extrapolate to zero concentration the values of the osmotic pressure per unit concentration (P/C) plotted against concentration (C). However, the relation of the data thus obtained to the structure of the original cellulosic material was often in error, because the important role played by the preliminary method of derivative formation was not recognized. In recent years it has been shown that valid conclusions can only be drawn from osmotic pressure data obtained with very dilute dispersions and then only when the results are extrapolated to infinite dilution.[113] Recognition of these prerequisites for obtaining valid data has led to the development of special methods for derivative formation and sensitive equipment for measuring the pressures developed.[114] Although it has been found that the limiting value of the ratio of osmotic pressure to concentration for a given derivative, found by extrapolation to zero concentration, is the same for all solvents, various derivatives give different maximum average degrees of polymerization. Thus the average degree of polymerization of nitrocellulose was found to be 770,[114] 430,[115] and 1,600[116]; of cellulose acetate, 600,[114] 160,[117] 310,[118]

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[111] J. Duclaux and E. Wollman, *Compt. rend.*, **152**, 1580 (1911).

[112] W. Ostwald, *Kolloid-Z.*, **23**, 68 (1918); **49**, 60 (1929).

[113] K. H. Meyer, "High Polymers, Vol. 4, Natural and Synthetic High Polymers," Interscience Publishers, Inc., New York (1942).

[114] E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 153 (1935); H. Mark, "High Polymers, Vol. 2, Physical Chemistry of High Polymeric Systems," Interscience Publishers, New York (1940); S. R. Carter and B. R. Record, *J. Chem. Soc.*, 660 (1939); R. E. Montonna and L. T. Jilk, *J. Phys. Chem.*, **45**, 1374 (1941); B. H. Zimm and I. Myerson, *J. Am. Chem. Soc.*, **68**, 911 (1946).

[115] A. Dobry, *J. chim. phys.*, **32**, 50 (1935).

[116] H. Staudinger and G. V. Schulz, *Ber.*, **68**, 2320 (1935).

[117] A. M. Sookne, H. A. Rutherford, H. Mark and M. Harris, *J. Research Natl. Bur. Standards*, **29**, 123 (1942); A. M. Sookne and M. Harris, *ibid.*, **30**, 1 (1943); *Ind. Eng. Chem.*, **37**, 475, 478 (1945).

[118] W. Herz, *Cellulosechem.*, **15**, 95 (1934).

380,[119] and 260[120]; of benzylcellulose, 84[121]; of ethylcellulose, 270[122]; and of methylcellulose, 450.[116]

It is thus evident that osmotic pressure measurements of cellulose derivatives give variable results depending upon the method of derivative formation and upon the derivative prepared. Although osmotic pressure measurements have been used as a primary standard in the calibration of other physical methods for determining the degree of polymerization of cellulosic materials, they should not be relied upon to give valid information concerning the degree of polymerization of the original cellulose component of native cellulosic materials. However, in spite of the difficulties attending the measurement of the small osmotic pressures developed by highly polymerized cellulosic derivatives, Schulz[123] claims that, with proper treatment of the data, valid conclusions can be drawn. Staudinger[124] substantiates this conclusion, stating that an average degree of polymerization as high as 6,000 can be determined with accuracy.

b. Ultracentrifuge Measurements. The use of the ultracentrifuge for determining the mass, size, shape and heterogeneity of the dispersed particle phase of highly polymerized materials in various solvents has been extensively investigated by Svedberg.[125]

The degree of polymerization of cellulosic materials dispersed in a dispersing agent such as cuprammonium hydroxide solution may be calculated from either (a) sedimentation velocity data, or (b) sedimentation equilibrium data. In following the former procedure a determination of the rate of movement of the sedimenting boundary under a high centrifugal force and the extent of diffusion across the boundary is determined. The calculated degree of polymerization is then obtained from the equation:

$$D.P. = \frac{RTs}{162D(1 - V\rho)} \tag{7}$$

in which R is the gas constant; T, the absolute temperature; s, the sedimentation constant per unit centrifugal force; D, the diffusion constant; V, the partial specific volume of the material; and ρ, the density of the

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(119) R. Obogi and E. Broda, *Kolloid-Z.*, **69**, 172 (1934).

(120) A. Dobry, *Bull. soc. chim.*, [5] **2**, 1882 (1935).

(121) R. O. Herzog and H. M. Spurlin, *Z. physik. Chem.*, *Bodenstein-Festband*, 239 (1931); S. A. Glikman, *J. phys. chem.* (U. S. S. R.), **12**, 81 (1938).

(122) I. Okamura, *Cellulosechem.*, **14**, 135 (1933).

(123) G. V. Schulz, *Z. physik. Chem.* **A158**, 237 (1932); **A159**, 374 (1932); **A160**, 409 (1932); **A176**, 317 (1936); *Angew. Chem.*, **49**, 863 (1936).

(124) H. Staudinger, *Papier-Fabr.*, **36**, 373 (1938).

(125) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge, Int. Ser. of Monographs on Physics," Oxford University Press, Oxford (1940).

solvent. The accuracy of the method depends upon the correct evaluation of the sedimentation and diffusion constants for the system.

The sedimentation equilibrium procedure, on the other hand, depends upon maintaining a centrifugal force just sufficient to give a measurable concentration change in relation to the height of the cell. The establishment of this equilibrium between the settling and diffusion rates of the dispersed phase enables the degree of polymerization of the material to be calculated from the equation:

$$D.P. = \frac{2RT \ln (c_2/c_1)}{162(1 - V\rho)\omega^2(x_2^2 - x_1^2)} \tag{8}$$

in which c_1 and c_2 are the concentrations at distances x_1 and x_2, respectively, from the center of rotation, and ω is the angular velocity of the dispersion.

Using the sedimentation velocity procedure, Stamm[126] found the average degree of polymerization of purified cotton linters dispersed in cuprammonium hydroxide solution to be 346. It was subsequently shown by Svedberg,[125] however, that reliable values for the diffusion constant could not be obtained in the ultracentrifuge and consequently the results of Stamm are probably in error.

Kraemer,[127] in following the sedimentation equilibrium procedure, found the calculated degree of polymerization of purified cotton linters, which were part of the same sample used earlier by Stamm,[126] to be 1852, using cuprammonium hydroxide solution as the dispersing agent. Similarly, regenerated cellulosic material gave a *D.P.* of 555 to 679. Several cellulose acetate fractions dispersed in acetone gave *D.P.* values of 331 to 590, and ethylcellulose dispersed in dioxane gave a calculated degree of polymerization of 502. The calculated degree of polymerization of several fractions of methylcellulose dispersed in water was found by Signer and Tavel[128] to vary between 69 and 186, employing the sedimentation equilibrium procedure. Further data obtained showed that the average length of the dispersed particles in the fractions varied between 410 Å and 1040 Å, whereas the diameter changed very little, namely, from 8.6 Å to 9.2 Å.

The polydispersity of the cellulosic material may be readily determined by the sedimentation equilibrium procedure from the relative distribution of the dispersed particles from the axis of rotation. The

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(126) A. J. Stamm, *J. Am. Chem. Soc.*, **52**, 3047 (1930).

(127) E. O. Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938); E. O. Kraemer and W. D. Lansing, *Nature*, **133**, 870 (1934); E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 153 (1935).

(128) R. Signer and P. V. Tavel, *Helv. Chim. Acta*, **21**, 535 (1938).

average degree of polymerization on either a number or weight basis can thus be obtained.

It will be observed in general that the degree of polymerization of degraded cellulosic materials and derivatives, calculated from ultracentrifuge data, agree reasonably well with the calculated values obtained by chemical methods. However, when dealing with native cellulosic materials, it is doubtful that any rigid correlation will be found between the various methods, for in most cases the values for particle mass and molecular weight, in the usual sense of the terms, may be entirely different. This point of view finds justification in the more recent work of Gralén and Svedberg,[129] who have shown that for reliable results to be obtained, the sedimentation and diffusion values of cellulosic materials dispersed in cuprammonium hydroxide solution must be obtained in the absence of oxygen and that the measurements must be extrapolated to zero concentration just as in the case of osmotic pressure measurements. With this improved technique, the calculated degree of polymerization of unbleached cotton linters was found to be 9,200; untreated cotton fiber, 6,200; nettle fiber, 10,800; ramie, 11,300; and sulfite wood pulp, 2,900. Evidence was also presented which indicated that considerable curling of the molecules occurred in the dispersions, and that there was no linear relation between relative viscosity and degree of polymerization or molecular weight. It thus appears that it is probably more in order to consider the values obtained as average dispersed particle weights rather than true average molecular weights, although the subject cannot in any sense be considered closed.

c. Viscosity Measurements. Of the various physical methods used for determining the degree of polymerization of cellulosic materials and derivatives, the viscosity method is the simplest and hence the most widely used. The empirical relation of Staudinger[109] is expressed in the equation

$$(D.P.)(K_m) = \frac{\eta_{sp}}{c} = \frac{\eta_r - 1}{c} \qquad (9)$$

in which η_r is the viscosity of a dispersion of a polymeric material relative to that of the solvent; c is the concentration of the solute in grams per liter; η_{sp} is the specific viscosity, or the increase in relative viscosity due to the solute; K_m is the proportionality constant for the particular polymer-homologous series in a given solvent, and $D.P.$ is the calculated degree of polymerization of the polymeric material. On solving this equation for K_m, it will be noted that if the degree of polymerization of any member of a polymer-homologous series is known, the value of the proportionality constant may be determined from data concerning the

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(129) N. Gralén and T. Svedberg, *Nature*, **152**, 625 (1943).

specific viscosity per unit concentration. In the case of derivatives of cellulosic materials dispersed in organic solvents, the K_m values were determined, as previously mentioned, on very highly degraded products, but the use of the osmotic pressure method of Schulz[123] has apparently extended the range to less highly degraded products. From the K_m values thus found, it is claimed that equation 9 can be used for calculating the degree of polymerization of various derivatives of cellulosic materials over the complete viscosity range. It may thus be seen that the viscosity method is not an absolute method for determining the degree of polymerization but is a relative method for expressing changes in viscosity in a systematic manner, and with considerable accuracy. The K_m values vary with the solvent and with the cellulosic material or derivative over the range of 5 to 21 $\times 10^{-4}$. In the case of cellulosic materials dispersed in cuprammonium hydroxide and in phosphoric or sulfuric acid, it is assumed that the degree of polymerization is the same as that of the acetate or nitrate derivative derived therefrom. From this relationship the K_m value of cellulosic materials has been calculated to be 5×10^{-4} when dispersed in cuprammonium hydroxide solution, and 18 to 21 $\times 10^{-4}$ in phosphoric or sulfuric acid solution.

Just as osmotic pressure and ultracentrifugal measurements must be made at high dilution, so the viscosity of dispersions of cellulosic materials and derivatives must be determined at very high dilution. The recognized practice at present is to obtain three or more η_{sp}/c values for a particular system at relatively low concentrations and plot the logarithms of these values against the concentration c, expressed in grams per liter. The intercept values of η_{sp}/c, obtained by extrapolation to zero concentration, and substituted in equation 9, give a very reliable calculated degree of polymerization. The η_{sp}/c values obtained in this manner are usually of about the same magnitude or identical with those obtained below what has been called the critical concentration. The critical concentration is the concentration below which a linear relationship exists between the logarithm of η_{sp}/c and c, and according to Staudinger,[130] is dependent only on the chain length of the high polymer molecule. From calculations based on concentration-viscosity data extrapolated to infinite dilution, and relating to cellulosic materials dispersed in cuprammonium hydroxide solution, it was found that the volume of free rotation of the average molecule should be considered like unto a disk formed by the rotation of the molecule about its width axis in the plane of the length axis. However, studies made under variable shear conditions by Stamm and Cohen[131] indicated that the limiting concentration depended upon

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(130) H. Staudinger, *Trans. Faraday Soc.*, **29**, 18 (1933).

(131) A. J. Stamm and W. E. Cohen, *J. Phys. Chem.*, **42**, 921 (1938).

the velocity of shear as well as the chain length, and that the molecular volume of free rotation corresponded more closely to that of a sphere whose diameter was equal to the average chain length of the molecule.

The calculated degree of polymerization of several cellulosic materials (K_m value, 5×10^{-4}) and nitrate derivatives (K_m value, 11×10^{-4}) prepared therefrom, have been reported by Staudinger[132] as follows: raw cotton linters, 2,700; the corresponding nitrate derivative, 3,200; cotton linters slightly bleached, 1,600; the corresponding nitrate, 3,500. Golova and Ivanov,[133] on the other hand, state that the presence of oxygen in the cuprammonium hydroxide dispersions of cellulosic materials is responsible for the low calculated degree of polymerization usually encountered. When the viscosity determinations were made in the presence of nitrogen which had been carefully purified to eliminate oxygen, the calculated *D.P.* of raw cotton was found to be 9,500 to 10,000. Even this value was considered low due to the presence of traces of oxygen. To obtain the true *D.P.*, a graphic extrapolation to zero oxygen content was made which showed that the native cellulosic material was in the *D.P.* range of 15,000. It will be recalled that Svedberg found values of this order when cuprammonium dispersions of cellulosic materials were centrifuged in a nitrogen atmosphere.

Although osmotic pressure measurements made on derivatives of cellulosic materials serve as the basis for calculating the K_m value used in the Staudinger equation for calculating the degree of polymerization of cellulosic materials and derivatives from viscosity measurements, the two methods give identical results only with homogeneous materials. Nevertheless, the former method, which gives the number average degree of polymerization, has been used in obtaining the K_m values of a polydispersed material used in the latter method, which supposedly gives the weight average degree of polymerization. Kraemer[127] has avoided this error by using the ultracentrifuge and determining the K_m values from sedimentation equilibrium data, which give weight average degrees of polymerization (or particle weights) as does the viscosity method. The K_m values determined in this manner and expressed in the same way as those of Staudinger were found to be for cellulosic materials in cuprammonium hydroxide solution, 3.8×10^{-4}; for nitrocellulose dispersed in acetone, 4.35×10^{-4}; and for ethylcellulose dispersed in dioxane, 3.33×10^{-4}. It will be noted that the K_m values are much smaller and less variable than those found by Staudinger for the same systems. Thus, when used in calculating the degree of polymerization

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(132) H. Staudinger and R. Mohr, *Ber.*, **70**, 2307 (1937).

(133) O. P. Golova and V. I. Ivanov, *Bull. acad. sci. U.R.S.S.*, *Classe sci. chim.*, 279 (1945); *C. A.*, **40**, 1653 (1946).

from viscosity data, the K_m values of Staudinger will give a considerably lower result than the K_m values of Kraemer. Kraemer preferred to use a modification of Staudinger's equation for calculating the degree of polymerization of polymeric materials, namely,

$$D.P. = \lim_{c \to 0} \left(\frac{\eta_r - 1}{c} \right) = K[\eta] \tag{10}$$

in which $[\eta]$ is a new term, the intrinsic viscosity, defined as the limiting value of the viscosity increment $(\eta_r - 1)$, or the equivalent term, η_{sp} divided by the concentration of the solute, c (expressed in grams per 100 ml.), as c approaches zero. For different systems, the values of the proportionality constant, K, in this equation are positive integers because of a reciprocal relation with the K_m values of Staudinger. The terminology used in the literature for discussing viscosity functions has become so confusing that consideration should be given to the adoption of constructive suggestions which tend to clarify the subject, such as those offered by Cragg.[134] The commonly quoted K values found by Kraemer are as follows: cellulosic material dispersed in cuprammonium hydroxide solution, 260; nitrocellulose in acetone, 270; secondary cellulose acetate in acetone, 230; and ethylcellulose in dioxane, 300.

The validity of the relation expressed in equation 10 has been questioned from time to time and it has been demonstrated[135] that the more general equation,

$$\left(\frac{\eta_{sp}}{c} \right)_{c=0} = [\eta] = KM^a \tag{11}$$

in which a is a constant with values between 0.5 and 2, and M is the molecular weight, conforms more closely to experimental data. Both constants, K and a, must be determined empirically from the graph of $[\eta]$ against M obtained from polymer samples which are molecularly homogeneous. Since M is not known with absolute certainty it is evident that true molecular weights, or degrees of polymerization of polydispersed cellulose or cellulose derivatives cannot be deduced from viscosity data. The equation must first be tested on homogeneous cellulose or cellulose derivative dispersions before it can be accepted as adequate.

The need for greater accuracy in determining the intrinsic viscosity, $[\eta]$, of cellulose dispersed in cuprammonium hydroxide solution led

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(134) L. H. Cragg, *J. Colloid Sci.*, **1**, 261 (1946).

(135) P. J. Flory, *J. Am. Chem. Soc.*, **65**, 372 (1943).

Martin[136] to develop, by empirical means, the equation

$$\log \frac{\eta_{sp}}{c} = \log [\eta] + k[\eta]c \tag{12}$$

in which c is the concentration expressed in grams per 100 grams of solution and k is a constant characteristic of a given polymeric series dispersed in a given solvent. For cellulose dispersed in cuprammonium hydroxide solution the relation of this equation to other viscosity concentration equations has been summarized by Martin[136] and by Huggins.[137] As pointed out by Huggins, the value of k for a given polymer-solvent combination can be deduced from a series of measurements on a polymer sample at different concentrations. For this purpose the polymer need not be homogeneous, as regards molecular weight. A convenient procedure is to plot η_{sp}/c against η_{sp} and to draw the best straight line through the experimental points. The ratio of the slope to the intercept (at $c = 0$) is k. Once this constant has been determined, a single viscosity measurement suffices to determine $[\eta]$, and hence the molecular weight or degree of polymerization, if the curve relating intrinsic viscosity and molecular weight is known. In the absence of this latter data, the value of K in equations 10 and 11 must be assumed, or derived from some other data such as osmotic pressure measurements. Using this latter procedure it has been shown[138] that the value of K may be determined from the relationship which exists between the intrinsic viscosity $[\eta]$, and the number average molecular weight $\overline{M}_n$, or degree of polymerization, as determined from osmotic pressure measurements. In this case, the algebraically extrapolated intrinsic viscosity $[\eta]$ values were determined by using equation 12 and applying the method of least squares. Treating the data as though they conformed with equation 11, a value of $a = 1.03$ was obtained, also by the method of least squares. At higher values of calculated molecular weights, such as those of cellulosic materials dispersed in cuprammonium hydroxide solution,[110] the value of $[\eta]$ may increase more rapidly than $\overline{M}_n$, in which case the value of a in equation 11 becomes less than unity.

From viscosity measurements and the use of K values (equation 10) obtained from sedimentation equilibrium measurements, Kraemer[127]

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(136) A. F. Martin, Abstracts of Papers, 103rd Meeting of the American Chemical Society, Memphis, Tenn., p. 1C (1942); see also "Cellulose and Cellulose Derivatives," E. Ott, editor, pp. 966–971, Interscience Publishers, Inc., New York (1943).

(137) M. L. Huggins *in* "Cellulose and Cellulose Derivatives," E. Ott, editor, pp. 950–955, Interscience Publishers, Inc., New York (1943).

(138) A. M. Sookne and M. Harris, *Ind. Eng. Chem.*, **37**, 475 (1945).

found the following calculated degrees of polymerization: native cellulosic material, 3,500; purified cotton linters, 1,000–3,000; wood pulps, 600–1,000; secondary cellulose acetates, 175–360; secondary nitrocellulose, 175; plastic nitrocellulose, 500–600; dynamite nitrocellulose, 3,000–3,500; ethylcellulose, 540. The ability to transform a secondary cellulose acetate to a regenerated cellulose and this back to a secondary cellulose acetate without changing the calculated degree of polymerization, when using weight average *K* values in the appropriate solvent, convinced Kraemer that cellulosic materials and derivatives are dispersed as elongated particles which should be considered molecules, as indicated by Staudinger. The possibility that the ratio of Kraemer's *K* values for cellulosic materials dispersed in cuprammonium solution and for the corresponding secondary acetate in acetone is not always a constant may be inferred from the work of Howlett and Urquhart.[139] Some doubt exists, therefore, as to the validity of Kraemer's conclusions.

Despite the evidence presented, the opinion that cellulosic materials, and perhaps cellulose derivatives, are molecularly dispersed in various dispersing agents is not unanimous. This dissenting opinion is based upon data obtained by more or less conventional methods, but the conclusions drawn therefrom depart somewhat from those of the Staudinger school. Among investigators who have suggested that cellulose and cellulose derivatives exist in various dispersing agents as preformed micelles or particles, should be mentioned Mark,[140] Meyer,[113] Hess and coworkers,[141] Lieser,[142] Farr,[143] Sisson,[144] and Compton.[145] The internal structure of the organized entities has not been definitely established but it is likely composed of relatively low *D.P.* chain molecules held together by unique primary valence bonds, Van der Waal's forces, hydrogen bonding, dipole interaction, or some other undetermined force. The similarity of this concept to that recently proposed by Pacsu,[92a] is readily evident. On the other hand, evidence for the association of cellulose, or cellulose derivative, molecules in various dispersing agents to form aggregates of a different type than those preformed in the

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(139) F. Howlett and A. R. Urquhart, *J. Textile Inst.*, **37**, T89 (1946).

(140) H. Mark, "High Polymers, Vol. 2, Physical Chemistry of High Polymeric Systems," pp. 273–295. Interscience Publishers, Inc., New York (1940).

(141) K. Hess, W. Wergin, H. Kiessig, W. Engel and W. Philippoff, *Naturwissenschaften*, **27**, 622 (1939). See W. Wergin, *Protoplasma*, **32**, 116 (1939); W. Philippoff, *Angew. Chem.*, **49**, 855 (1936).

(142) T. Lieser, *Ber.*, **74**, 708 (1941).

(143) Wanda K. Farr, *J. Phys. Chem.*, **41**, 987 (1937).

(144) W. A. Sisson, *Contrib. Boyce Thompson Inst.*, **10**, 113 (1938).

(145) J. Compton, *Ind. Eng. Chem.*, **31**, 1250 (1939); *J. Am. Chem. Soc.*, **60**, 2823 (1938).

plant cell, has also been reported by Ekenstam,[145a] McBain and Scott,[98] and Bergek and Ouchterlony.[145b]

d. Diffusion Measurements. From diffusion measurements, Herzog and Krüger[146] reported the degree of polymerization of cellulose dispersed in cuprammonium hydroxide solution to be 3086. This value was doubtless fortuitous in the light of our present knowledge concerning dispersions of cellulose. These workers employed a relatively high concentration, one per cent, and assumed the cellulose particles to be spherical in using the Einstein diffusion equation,

$$D = \frac{RT}{N} \times \frac{1}{6\eta\pi r} \tag{13}$$

in which D is the diffusion constant; R, the gas constant; T, the absolute temperature; N, Avogadro's number; η, the viscosity of the solvent; and r, the radius of the diffusing particle. Using somewhat more dilute dispersions, Stamm[126] reported the degree of polymerization of cotton linters to be 346, a value admittedly in error because of the technique employed.

Diffusion data on methylcellulose fractions, obtained by Polson,[147] combined with the ultracentrifuge sedimentation data, obtained by Signer and Tavel,[128] when substituted in equation 7, gave calculated degrees of polymerization which agreed with values obtained from sedimentation equilibrium data alone. The chain length of the materials used was very small, being in the range of 69 to 186 methylated anhydro-D-glucose units.

e. Light Scattering Measurements. When light is passed through a dispersion of a derivative of a cellulosic material in an organic solvent, a typical Tyndall beam may be observed. The intensity of this scattered light depends upon the microscopic heterogeneity of the dispersion. Through the work of Debye[148] it was shown that by measuring the turbidity and the refractive indices of the solvent and the solution the molecular weight, or *D.P.*, of a dispersed substance could be estimated directly, using equations 14, 15 and 16.

$$\tau = \frac{32\pi^3\mu_0^2(\mu - \mu_0)^2}{3n\lambda^4} \tag{14}$$

$$\frac{Kc}{\tau} = \frac{1}{M} \tag{15}$$

(145*a*) A. H. Ekenstam, *Ber.*, **69**, 549 (1936).

(145*b*) T. Bergek and T. Ouchterlony, *Svensk Papperstidn.*, **49**, 470 (1946).

(146) R. O. Herzog and Deotata Krüger, *Kolloid-Z.*, **39**, 250 (1926).

(147) A. Polson, *Kolloid-Z.*, **83**, 172 (1938).

(148) P. Debye, *J. Applied Phys.*, **15**, 338 (1944). See P. P. Debye, *ibid.*, **17**, 392 (1946).

In these τ is the turbidity; μ_0, the refractive index of the solvent; μ, the refractive index of the solution; λ, the wave length of the light used; n, the number of particles per cubic centimeter; N, Avogadro's number; M, the molecular weight; c, the concentration in grams per cubic centimeter and

$$K = \frac{32\pi^3\mu_0{}^2}{3N\lambda^4}\left(\frac{\mu - \mu_0}{c}\right)^2. \tag{16}$$

A similar equation (17), obtained by Doty, Zimm and Mark,[149] relates the turbidity (actually, the extinction coefficient in cm.$^{-1}$ due to concentration fluctuations, which is the principal cause of heterogeneity) to: the refractive index, n; the concentration, c; the refractive index increment $(\partial n/\partial c)$; and the molecular weight, M, of the solute.

$$\frac{32\pi^3 n^2(\partial n/\partial c)^2}{3\lambda^4 N_0}\frac{c}{\tau} = H\frac{c}{\tau} = \frac{1}{M} + \frac{2B}{RT}c \tag{17}$$

In equation 17, λ represents the wave length of the monochromatic light used, N_0 is Avogadro's number, and B, which measures the deviation from van't Hoff's law, is the coefficient of the last term (c^2) in the expression for osmotic pressure $\left(\pi = RT\frac{c}{M} + Bc^2\right)$. H denotes factors which are constant for a given system.

As a result of the dissymmetry of the long-chain molecules, a correction factor must be applied to account for intramolecular interference. This correction factor is equal to the ratio of the intensity of the light scattering at 90° in the absence of intramolecular interference to the observed intensity. Taking this into consideration, it was found, in the case of several reasonably homogeneous fractions of cellulose acetate, that good agreement was obtained between the calculated degree of polymerization, or molecular weight, and values obtained by osmotic pressure and viscosity measurements.[150] This, of course, could only be true if the fractions gave near identical number and weight average degrees of polymerization.

Although the method has considerable merit, the greatest difficulty in its use arises from the large discrepancies caused by the presence of small amounts of impurities. Even in the work thus far reported, there is no proof that the dispersions were optically clean.

f. X-ray Diffraction Measurements. Unlike other physical methods for determining the degree of polymerization of cellulosic materials,

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(149) P. M. Doty, B. H. Zimm and H. Mark, *J. Chem. Phys.*, **12**, 144 (1944); **13**, 159 (1945).

(150) R. S. Stein and P. Doty, *J. Am. Chem. Soc.*, **68**, 159 (1946).

X-ray diffraction measurements may be made on the fibrous material. Although the method is limited in application, it does enable definite statements to be made about the minimal length of the crystalline fiber component. Thus the minimal length of the anhydro-D-glucose polymer chain in ramie was found to be about 1000 Å, and in wood, about 600 Å. From these data it may be calculated that the degree of polymerization is greater than 200,[151] and indeed may be found to be the approximate length of the basic molecular unit.[92a]

In a critical survey of the X-ray data obtained on cellulosic materials, Peirce,[151a] has proposed a modification of Meyer and Misch's,[151b] essentially orthorhombic model of the spatial arrangement of the molecules in the cellulose crystallite. The proposed modification satisfactorily accounts for the observed reflections and distortions which are foreign to orthorhombic symmetry, by rotating the primary hydroxyl group on one side of one of the two sets of molecular chains to form an internal, chelate bond with two oxygen atoms of the same chain. Hydroxyl groups undergoing chelation are those that are on alternate units, or on one side only, of one of the sets of molecular chains. This slightly distorted orthorhombic cell is based on a pyranose ring with its five carbon atoms nearly coplanar. Extension of this concept of molecular coordination also leads to a logical explanation for the conversion of native cellulose into a product of lower free energy content, namely, regenerated or mercerized cellulose.

V. POLYDISPERSITY OF CELLULOSE

The polydispersity of native cellulose has not been definitely shown but when derivatives of cellulose are dispersed in various solvents, polydispersity can definitely be demonstrated. As indicated earlier,[125,126,127] the most satisfactory method of separating particles of various weights or sizes is by use of the ultracentrifuge. However, because of the scarcity of equipment of this type, other methods have been used. When the nature of the degrading reaction is known and the assumptions made by Kuhn[80,81,82] are followed, the randomness or heterogeneity existing in a given sample may be calculated mathematically. However, the assumptions which Kuhn has made cannot be entirely justified if groups other than those present in a normal anhydro-D-glucose polymer are included

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(151) J. Hengstenberg and H. Mark, *Z. Krist.*, **69**, 271 (1929); K. Hess and C. Trogus, *Naturwissenschaften*, **18**, 437 (1930); K. Hess, C. Trogus, L. Akim and I. Sakurada, *Ber.*, **64**, 408 (1931).

(151*a*) F. T. Peirce, *Trans. Faraday Soc.*, **42**, 545, 560 (1946).

(151*b*) K. H. Meyer and L. Misch, *Ber.*, **70**, 266 (1937); *Helv. Chim. Acta*, **20, 232** (1937).

in the structure of the cellulose molecule. Fortunately, fractionation of dispersions of cellulose derivatives may be achieved. The method employed is based on the differential dispersibility of various particle sizes or weights, in various solvents or solvent mixtures. The separation of purified cellulosic materials into α-, β- and λ-cellulose by the differential dispersibility in 17.5% caustic at 20°C. has been previously referred to. In general, the λ-component is composed of material with a degree of polymerization of about 10, whereas that of the β-fraction covers the range 10 to 200, being largely 140 to 200.[152] The insoluble α-cellulose component consists predominantly of cellulosic material with a calculated degree of polymerization equal to or greater than 200. No information concerning the polydispersity of α-cellulose is thus possible by this means, but the principle of fractionation is demonstrated.

Highly degraded cellulosic materials, such as viscose rayon, *D.P.* 250 to 350, may be dispersed in 8–12% caustic at low temperatures. Upon increasing the temperature in a stepwise manner, the dispersed material may be fractionally precipitated.[153] This method of fractionation is of particular interest since viscosity data indicate that the state of dispersion of degraded cellulosic materials in caustic solution and in cuprammonium solution is similar.[154]

The fractionation of cellulosic materials dispersed in cuprammonium solution has usually been accompanied by an amount of degradation sufficient to render the results questionable. However, Battista and Sisson,[155] using acetone and *n*-propyl alcohol as precipitants, were able to resolve viscose rayon yarn (*D.P.* 490) dispersed in cuprammonium solution into fractions varying in average degree of polymerization from 535 to 142, and from 615 to 132, respectively.

The fractionation of cellulosic materials dispersed in aqueous solutions of ethylenediamine saturated with cupric hydroxide has been achieved by the stepwise addition of sulfuric acid, according to Straus and Levy.[156]

By careful nitration of cellulosic materials with nitric acid in the presence of phosphoric acid and phosphoric anhydride, a minimum of degradation occurs.[132] According to Schieber,[157] the resulting cellulose nitrate may be separated by stepwise dispersion with successively richer solvent mixtures into a series of fractions of varying chain lengths.

(152) R. E. Dörr, *Angew. Chem.*, **53**, 13, 292 (1940).

(153) R. Neumann, R. Obogi and S. Rogowin, *Cellulosechem.*, **17**, 87 (1936).

(154) O. Eisenhut, *Melliand Textilber.*, **20**, 625 (1939).

(155) O. A. Battista and W. A. Sisson, *J. Am. Chem. Soc.*, **68**, 915 (1946).

(156) F. L. Straus and R. M. Levy, *Paper Trade J.*, **114**, 31 (Jan. 15, 1942); **114**, 33 (Apr. 30, 1942).

(157) W. Schieber, *Papier-Fabr.*, **37**, 245 (1939).

This method has been applied to the determination of the chain-length distribution in wood cellulose at various stages of purification and to purified wood pulp during the various steps of viscose rayon manufacture.[18] In general, it was found that purification decreased the distribution range as the maximum calculated degree of polymerization decreased. The distribution range in the original wood (western hemlock) was found to be from 0 to 3,000, with two distinct peaks being present at an average *D.P.* of 70 and 2,000 to 2,500. The first peak corresponds to the hemicelluloses which are present and largely removed by subsequent purification. The second peak, accounting for about 70% of the total, is largely cellulose. The sharpness of the chain length distribution is greatest for regenerated filaments from the purified wood pulp viscose, the range being from zero to about 700. The chain-length distribution of cellulose in cotton linters follows the same pattern as that of wood cellulose during purification, except that the presence of the hemicellulose fraction is obscured because of the preponderence of cellulose. The chain-length distribution of regenerated cellulose filaments from cotton linters viscose is somewhat sharper than that obtained from wood pulp, but the *D.P.* range is about the same, 0 to 750.

Using a somewhat different technique, Spurlin[158] fractionally precipitated cellulose nitrate from acetone solution with heptane. From the large number of fractions found, a rather broad chain-length distribution curve was obtained. It was found that the folding endurance of films prepared from certain fractions was superior to that of the original cellulose nitrate or to blends of fractions having the same viscosity or calculated degree of polymerization. Below a certain sharply defined viscosity, or *D.P.* value, the films from the various fractions did not withstand folding.

A study of the fractional precipitation (with 95% alcohol) of a commercial secondary cellulose acetate (*D.P.* 194) dissolved in acetone was made by Harris and coworkers.[117] The *D.P.* values obtained from viscosity measurements ranged from 36 to 380. The lower fractions would not give coherent films but as the *D.P.* increased, the mechanical properties of the films improved up to a *D.P.* of 150; above this value improvement was slight. In general, the conclusions drawn by Spurlin in the case of cellulose nitrate were found to hold for cellulose acetate. However, Spurlin had only investigated weight-average degrees of polymerization and Harris was able to show, in the case of cellulose acetate fractions, that the number-average degree of polymerization was more closely related to mechanical properties. The use of osmotic pressure measurements as a means of determining the degree of polymerization

(158) H. M. Spurlin, *Ind. Eng. Chem.*, **30**, 538 (1938).

of cellulosic derivatives is thus to be preferred over viscosity measurements in work of this type.

Further studies by Badgley and Mark[159] on relatively homogeneous fractions of a secondary cellulose acetate obtained by controlled precipitation from acetone and methylcellosolve solutions (with methyl alcohol), showed that equations 9, 10 and 11, above, apply over relatively narrow ranges of *D.P.* Above a *D.P.* of about 380, equation 9 gave *D.P.* values higher than those obtained by osmotic pressure measurements. Modification of equation 11, to the form

$$[\eta] = KM^{a} - K'M^{2a} \tag{18}$$

where the tentative values of the constants are $a = 1$, $K = 2.09 \times 10^{-5}$, $K' = 0.315 \times 10^{-10}$, more perfectly fit the experimental data. The correlation of these measurements with the calculated axial ratios of the molecules indicated that in the region of lower *D.P.* the molecule is rather rigidly extended, but as the *D.P.* increases the molecules take on a more spherical shape. This change in shape occurs at a *D.P.* of about 300, and hence corresponds to the point at which deviation from equation 9 begins. The methods used in this work may be a means of determining the *D.P.* range of cellulose and cellulose derivatives, where micelle, or particle, dispersions end and molecular dispersions begin.

The heterogeneity or polydispersity of cellulosic derivatives may be estimated from the ratio of the degree of polymerization calculated from viscosity data $(D.P.)_W$ to the degree of polymerization calculated from osmotic pressure data $(D.P.)_N$.[158,159] When the ratio $(D.P.)_W/(D.P.)_N$ for a given fraction is equal to unity, the material is homogeneous; on the other hand, increasingly higher values of the ratio indicate an increasingly greater heterogeneity.

In view of the chain-length distribution of degraded cellulose and its derivatives, any practical measurements of the degree of polymerization must necessarily give average values only. Now, if W is the total weight of any single constituent in a mixture of an average *D.P.*, then $[W/D.P.]$ is the average number of molecules present; the combined effect produced by all the components is thus proportional to $\Sigma[W/D.P.]$, and the number-average *D.P.* may be written as follows,

$$(D.P.)_N = \frac{\Sigma W}{\Sigma[W/D.P.]} = \frac{\Sigma_{N_i} D.P._i}{\Sigma_{N_i}} \tag{19}$$

in which N_i is the number of molecules of a degree of polymerization, $D.P._i$. The weight-average degree of polymerization, on the other hand, may be calculated from equation 20.

[159] W. J. Badgley and H. Mark, *J. Phys. Colloid Chem.*, **51**, 58 (1947).

$$(D.P.)_W = \frac{\Sigma[W \times D.P.]}{\Sigma W} = \frac{\Sigma_{W_j} D.P._j}{\Sigma_{W_j}} \qquad (20)$$

In this W_i is the total weight of the fractions of a given molecular species, and the other symbols have the same meaning as in equation 19.

A third kind of average degree of polymerization, referred to as the "*Z*" or "*C*" average,[160] is obtained by treatment of the data according to equation 21.

$$(D.P.)_Z = \frac{\Sigma[W \times (D.P.)^2]}{\Sigma[W \times D.P.]} = \frac{\Sigma_{W_j}(D.P._j)^2}{\Sigma_{W_j} D.P.} \qquad (21)$$

From these equations, it may be seen that the number-average *D.P.* gives relatively more importance to the smaller molecules, whereas the weight-average *D.P.* favors the larger molecules. It may further be seen that end-group methods and osmotic pressure measurements give number-average degrees of polymerization whereas viscosity and light scattering measurements give weight-average values. Sedimentation data, obtained in the ultracentrifuge, give number, weight or "*C*" average degrees of polymerization values, whereas diffusion measurements give weight, and perhaps "*Z*" or "*C*," averages.[161]

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(160) W. D. Lansing and E. O. Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935); E. O. Kraemer, *J. Franklin Inst.*, **231**, 1 (1941).

(161) I. Jullander, *J. Polymer Sci.*, **2**, 329 (1947).

ISOTOPIC TRACERS IN THE STUDY OF CARBOHYDRATE METABOLISM

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I. Introduction*

Although isotopes have been extremely useful for tracing the course of metabolic reactions in plants, bacteria, and mammals, it should be recognized that many other techniques have contributed in great measure to our knowledge of metabolism. Balance studies carried out both *in vivo* and *in vitro* upon plants as well as animals have enabled the biologist and biochemist to amass a great deal of information concerning metabolic processes. In cases, however, where such techniques lead to equivocal results the isotope technique is of paramount value. To illustrate, the ingestion of acetate by an animal produces no apparent effect upon the biological synthesis of carbohydrate. Nevertheless, administration to animals of acetate labelled with C^{13} results in the formation of isotopic glycogen even though there has been *no net increase* in glycogen synthesis. This state of affairs could not have been predicted prior to the use of isotopes. Conversely, increased excretion of some product as a result of administering a test substance does not imply that the atoms of the test substance have themselves been excreted. The fasted, phlorizinized rat excretes an increased amount of urinary D-glucose when the amino acid alanine is administered; nevertheless, the "extra" D-glucose excreted contains very few of the carbon atoms originally furnished by the administered D,L-alanine.[1] It is in the elucidation of such apparent paradoxes that the isotopes have shown their worth.

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* Two excellent review articles dealing with various phases of this subject have been published by J. M. Buchanan and A. B. Hastings, *Physiol. Revs.*, **26**, 120 (1946); and H. G. Wood, *Physiol. Revs.*, **26**, 198 (1946).

II. Isotopic Techniques

The most widely used isotopes for the study of carbohydrate metabolism have been the radioactive isotopes of carbon, C^{11} and C^{14}, and the stable elements, heavy carbon C^{13} and deuterium H^2. Each isotope offers certain advantages and disadvantages. Although deuterium is available in highest concentration and is easily measured, it suffers the handicap of lability wherever active hydrogens are concerned. This is particularly evident in the case of carbohydrates. It is interesting, however, that deuterium bound to carbon in carbohydrates is relatively stable, and Stetten and Boxer[2,3] have made skillful use of this fact in studying the biological synthesis of glycogen from D-glucose and lactic acid.

C^{11}, which is prepared mainly by deuteron bombardment of a boron oxide target, emits positrons as well as γ-rays and can be readily measured with an outside Geiger counter. Its short half life, however, limits its use (Table I).

TABLE I

Isotopes of Carbon

Isotope	*Abundance, atoms, %*	*Half-life*	*Preparation*
C^{10}	—	8.8 sec.	${}_5B^{10}$ (p, n)${}_6C^{10}$
C^{11}	—	21 min.	${}_5B^{10}$ (d, n)${}_6C^{11}$
C^{12}	98.9	—	
C^{13}	1.1	—	Thermal diffusion Chemical exchange
C^{14}	—	5000 years	${}_7N^{14}$ (n, p)${}_6C^{14}$ ${}_6C^{13}$ (d, p)${}_6C^{14}$

Although C^{14} has not been extensively used as yet, it promises to become one of the most useful of the isotopes. First made in 1940 by Ruben and Kamen,[4] it has the advantage of a very long half-life and can be obtained having an appreciable specific radioactivity (3–6%). It emits electrons with essentially no γ-ray emission, and because of its weak radiation, must be measured with a thin mica-window counter or a suitable ionization chamber.

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(1) S. Gurin and D. W. Wilson, *Federation Proc.*, **1**, 114 (1942).

(2) D. Stetten, Jr., and G. E. Boxer, *J. Biol. Chem.*, **155**, 231 (1944).

(3) G. E. Boxer and D. Stetten, Jr., *J. Biol. Chem.*, **155**, 237 (1944).

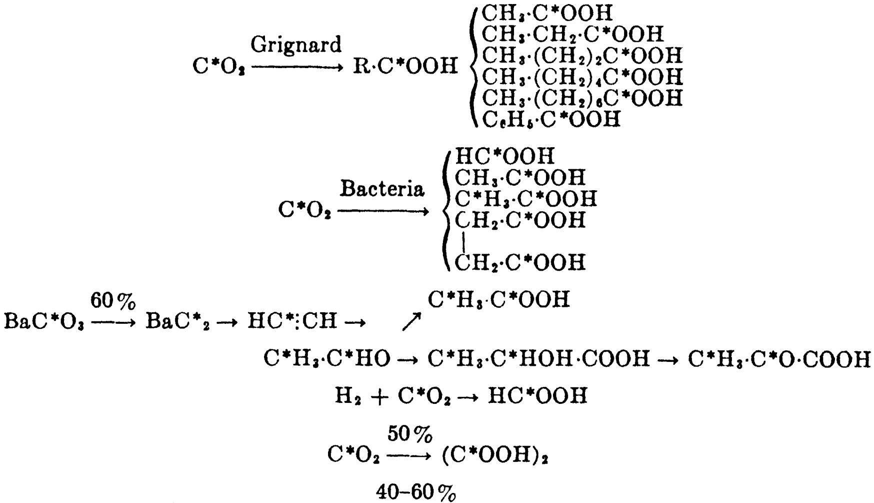
(4) S. Ruben and M. D. Kamen, *Phys. Rev.*, **57**, 549 (1940).

The stable isotope of carbon, C^{13}, is obtained in the form of methane, cyanide or a carbonate. This isotope is available in larger quantities than are the radioactive carbons although highly enriched material is difficult to obtain. Measurements are readily and accurately made with a mass spectrometer. The usual procedure involves combustion of an organic substance containing C^{13} by the wet oxidation procedure of Van Slyke and Folch[5] or by the usual combustion methods followed by purification of the resulting CO_2. The sample of CO_2 is then analyzed by means of the mass spectrometer. It should be emphasized at this point that C^{13} analyses of necessity must be carried out on analytically pure substances since most contaminants contain carbon. This is particularly true of biological substances which have to be isolated from natural sources.

An excellent survey of methods employed in the handling and measurement of radioactive carbon is to be found in Kamen's[5a] recent book.

III. SYNTHETIC METHODS

The synthesis of organic substances containing isotopic carbon suffers from several limitations. The short half-life of C^{11} necessitates very rapid synthesis while the small amounts of C^{13} and C^{14} that are available restrict the syntheses to a very small scale (0.01–0.05 moles).



$$C^*O_2 \xrightarrow{\text{Grignard}} R{\cdot}C^*OOH \left\{ \begin{array}{l} CH_3{\cdot}C^*OOH \\ CH_3{\cdot}CH_2{\cdot}C^*OOH \\ CH_3{\cdot}(CH_2)_2C^*OOH \\ CH_3{\cdot}(CH_2)_4C^*OOH \\ CH_3{\cdot}(CH_2)_6C^*OOH \\ C_6H_5{\cdot}C^*OOH \end{array} \right.$$

$$C^*O_2 \xrightarrow{\text{Bacteria}} \left\{ \begin{array}{l} HC^*OOH \\ CH_3{\cdot}C^*OOH \\ C^*H_3{\cdot}C^*OOH \\ CH_2{\cdot}C^*OOH \\ \vert \\ CH_2{\cdot}C^*OOH \end{array} \right.$$

$$\begin{array}{l} BaC^*O_3 \xrightarrow{60\%} BaC^*_2 \rightarrow HC^*{:}CH \rightarrow \quad \nearrow C^*H_3{\cdot}C^*OOH \\ \qquad\qquad C^*H_3{\cdot}C^*HO \rightarrow C^*H_3{\cdot}C^*HOH{\cdot}COOH \rightarrow C^*H_3{\cdot}C^*O{\cdot}COOH \end{array}$$

$$H_2 + C^*O_2 \rightarrow HC^*OOH$$

$$C^*O_2 \xrightarrow{50\%} (C^*OOH)_2$$

$$C^*O_2 + 4K + NH_3 \xrightarrow{40\text{–}60\%} KC^*N + 2KOH + KH$$

FIG. 1.—Isotopic compounds prepared from C^*O_2.

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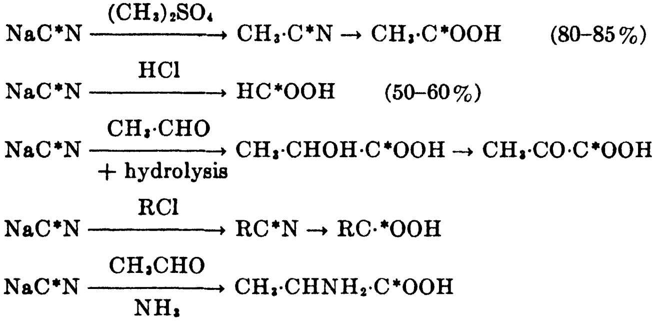
(5) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

(5a) M. D. Kamen, "Radioactive Tracers in Biology," Academic Press, N. Y. (1947).

Furthermore, many condensations occur in moderate or low yields so that it is frequently necessary to spend a considerable amount of time and effort to carry out what might seem to be relatively simple syntheses. In Figs. 1 and 2 are listed most of the syntheses of this type that have been reported. A few deserve particular mention.

Long[6] has prepared oxalic acid in 50% yield by introducing $C^{11}O_2$ into an evacuated flask containing molten potassium and sand. Acetic acid labelled with C^{14} in both carbons has been prepared by Barker and Kamen[7] from $Na_2C^{14}O_3$ by a bacterial synthesis. A total of 81% of the C^{14} was recovered in the acetic acid. Sakami, Evans and Gurin[8] have prepared ethyl acetoacetate labelled with C^{13} in the carbonyl and carboxyl groups; preparations of acetoacetate containing C^{13} located solely in the carboxyl or carbonyl group were also reported.



$$NaC^*N \xrightarrow{(CH_3)_2SO_4} CH_3{\cdot}C^*N \rightarrow CH_3{\cdot}C^*OOH \qquad (80\text{–}85\%)$$

$$NaC^*N \xrightarrow{HCl} HC^*OOH \qquad (50\text{–}60\%)$$

$$NaC^*N \xrightarrow[+\text{ hydrolysis}]{CH_3{\cdot}CHO} CH_3{\cdot}CHOH{\cdot}C^*OOH \rightarrow CH_3{\cdot}CO{\cdot}C^*OOH$$

$$NaC^*N \xrightarrow{RCl} RC^*N \rightarrow RC{\cdot}{}^*OOH$$

$$NaC^*N \xrightarrow[NH_3]{CH_3CHO} CH_3{\cdot}CHNH_2{\cdot}C^*OOH$$

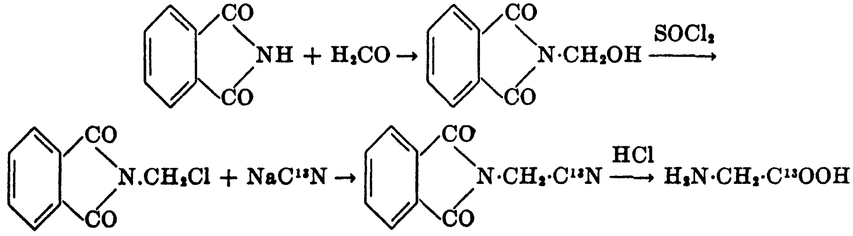
FIG. 2.—Isotopic compounds prepared from cyanide.

$$CH_3{\cdot}C^{13}OOC_2H_5 \rightarrow CH_3{\cdot}C^{13}O{\cdot}CH_2{\cdot}C^{13}OOC_2H_5$$

$$BrMgCH_2{\cdot}C^{13}OOC_2H_5 + CH_3{\cdot}COOCH_3 \rightarrow CH_3{\cdot}CO{\cdot}CH_2C^{13}OOC_2H_5$$

$$BrMgCH_2{\cdot}COOC_2H_5 + CH_3{\cdot}C^{13}OOCH_3 \rightarrow CH_3{\cdot}C^{13}OCH_2COOC_2H_5$$

Glycine labelled in the carboxyl group has been prepared[8] in 81% yield from $NaC^{13}N$.



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(6) F. A. Long, *J. Am. Chem. Soc.*, **61**, 570 (1939).

(7) H. A. Barker and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **31**, 219 (1945).

(8) W. Sakami, W. E. Evans and S. Gurin, *J. Am. Chem. Soc.*, **69**, 1110 (1947).

Harman, Stewart and Ruben[9] have prepared $C^{11}H_3I$ by the following steps. The yields are not reported.

$$C^{11}O_2 + H_2 \xrightarrow{B.\ coli} HC^{11}OOH \rightarrow HC^{11}OOCH_3$$

$$HC^{11}OOCH_3 + 2H_2 \xrightarrow{CuO \cdot CrO_3} C^{11}H_3OH \xrightarrow{HI} C^{11}H_3I$$

A number of new or modified syntheses have recently been reported. Catalytic reduction of CO_2 at high pressures has been employed to give excellent yields of isotopic methanol.[9a] Carbon dioxide can be easily converted to CO by a catalytic exchange method.[9b] Excellent yields of CO are readily obtained in this manner. The conversion of CO_2 to KCN has been improved with yields of 90-96 per cent reported.[9c]

C^{14} has been successfully introduced into the amino acids phenylalanine,[9d] tyrosine[9e] and methionine.[9f]

Ring A of cholestenone[9g] as well as of testosterone[9h] has been labeled with C^{14} by Turner while Heidelberger, Brewer and Dauben[9i] have synthesized isotopic 1,2,5,6-dibenzanthracene.

IV. CARBON DIOXIDE ASSIMILATION IN PLANTS

Since the subject of photosynthesis has been fully reviewed elsewhere[10,11,12] only the results obtained with isotopic tracers will be discussed in this report.

Ruben, Kamen and Hassid[13] exposed plants to an atmosphere of radioactive $C^{11}O_2$ and were subsequently able to separate a radioactive fraction containing alcoholic and carboxyl groups. In the presence of light, radioactive CO_2 was incorporated primarily into the non-carboxylic

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(9) D. Harman, T. D. Stewart and S. Ruben, *J. Am. Chem. Soc.*, **64**, 2293 (1942).

(9*a*) B. M. Tolbert, *J. Am. Chem. Soc.*, **69**, 1529 (1947).

(9*b*) J. T. Kummer, *J. Am. Chem. Soc.*, **69**, 2239 (1947).

(9*c*) R. B. Loftfield, Isotopes Branch, United States Atomic Energy Commission Circular C-3, June, 1947.

(9*d*) S. Gurin and A. M. Delluva, *Proc. Am. Soc. Exptl. Biol.*, **6**, 257 (1947).

(9*e*) J. C. Reid, *Science*, **105**, 208 (1947).

(9*f*) D. B. Melville, J. R. Rachele and E. B. Keller, *J. Biol. Chem.*, **169**, 419 (1947).

(9*g*) R. B. Turner, *J. Am. Chem. Soc.*, **69**, 726 (1947).

(9*h*) R. B. Turner, Isotopes Branch, United States Atomic Energy Commission Circular C-5, July, 1947.

(9*i*) C. Heidelberger, P. Brewer and W. G. Dauben, *J. Am. Chem. Soc.*, **69**, 1389 (1947).

(10) J. Franck and H. Gaffron, *Advances in Enzymology*, **1**, 199 (1941).

(11) H. Gaffron, *Biol. Rev. Cambridge Phil. Soc.*, **19**, 1 (1944).

(12) C. B. Van Niel, *Physiol. Revs.*, **23**, 338 (1943).

(13) S. Ruben, M. D. Kamen and W. Z. Hassid, *J. Am. Chem. Soc.*, **62**, 3443 (1940).

carbons whereas in the dark the plant yields a fraction containing radioactivity localized mainly in the carboxyl groups. Preliminary data obtained by diffusion and ultracentrifuge studies have suggested that this substance has a molecular weight ranging from 1000 to 1500.[14,15] No radioactivity was detected in the low molecular weight fractions. Investigation of the nature of this and related products is undoubtedly being actively pursued.

The first step in the assimilation of CO_2 is assumed by Ruben, Kamen and coworkers to involve a non-photochemical reaction, catalyzed enzymically and resulting in the addition of CO_2 to a large molecule. The process is initiated rapidly in the dark and gradually decreases in rate. These results suggest that it is reversible. Since no correlation could be found between the concentration of chlorophyll in the leaves and the CO_2 uptake, it is believed that chlorophyll is not concerned with this initial reaction. Chlorophyll is believed to catalyze a second reaction involving the reduction of the carboxylic compound to an alcohol or polyalcohol. This second reaction, believed to occur in the presence of light, is endothermic and irreversible.

$$RH + CO_2 \leftrightarrows R{\cdot}COOH \text{ (dark)} \qquad (1)$$

$$R{\cdot}COOH + H_2O \xrightarrow{\text{chlorophyll}} R{\cdot}CH_2OH + O_2 \text{ (light)} \qquad (2)$$

By analogy with a reversible CO_2 assimilation reaction known to occur in certain bacteria, Lipmann and Tuttle[16] have proposed a theory explaining how carbon chains may be progressively lengthened by a process involving phosphorylation, CO_2 assimilation and photoreduction.

$$R.COOH + \overset{\text{adenosine}}{\text{triphosphate}} \rightarrow R{\cdot}CO{\cdot}OPO_3^-$$

$$R{\cdot}CO{\cdot}OPO_3^- + CO_2 + H_2 \xrightarrow{\text{(photolytic)}} R{\cdot}CO{\cdot}COOH + HOPO_3^-$$

$$R{\cdot}CO{\cdot}COOH + H_2 \xrightarrow{\text{(photolytic)}} R{\cdot}CHOH{\cdot}COOH$$

The source of hydrogen is the photolytic cleavage of water with oxygen resulting as a by-product.

Ruben and Kamen[17] have also found that fresh yeast cells suspended in water will likewise assimilate CO_2.

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(14) S. Ruben and M. D. Kamen, *J. Am. Chem. Soc.*, **62**, 3451 (1940).

(15) S. Ruben, M. D. Kamen and L. H. Perry, *J. Am. Chem. Soc.*, **62**, 3450 (1940).

(16) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **154**, 725 (1944); **158**, 505 (1945).

(17) S. Ruben and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **26**, 418 (1940).

V. Carbon Dioxide Assimilation in Microorganisms

The assimilation of CO_2 by bacteria was first demonstrated by Wood and Werkman[18,19] who observed that during the fermentation of glycerol by propionic acid bacteria there was an over-all uptake of CO_2 and that the amount of product (succinic acid) formed corresponded to the CO_2 which was taken up. Since pyruvic acid was known to be present[20] it was proposed that pyruvic acid condenses with CO_2 to produce oxalacetic acid (Fig. 3, Wood-Werkman reaction) which is subsequently converted to succinic acid.

I. Wood-Werkman

A. $C^*O_2 + CH_3{\cdot}CO{\cdot}COOH \rightleftharpoons HOOC^*{\cdot}CH_2{\cdot}CO{\cdot}COOH$

II. Barker-Kamen

B. $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3{\cdot}COOH + 2CO_2 + 8H$

$2C^*O_2 + 8H \rightarrow C^*H_3{\cdot}C^*OOH + 2H_2O$

C. $2C^*H_3{\cdot}C^*OOH \rightleftharpoons HOOC^*{\cdot}C^*H_2{\cdot}C^*H_2{\cdot}C^*OOH$

$2C^*H_3{\cdot}C^*OOH \rightarrow C^*H_3{\cdot}C^*H_2{\cdot}C^*H_2{\cdot}C^*OOH$

III. Koepsell

$C^*O_2 + H_2 + CH_3{\cdot}CO(OPO_3H_2) \rightleftharpoons CH_3{\cdot}CO{\cdot}C^*OOH + H_3PO_4$

IV. Lipmann

$HC^*OOH + CH_3{\cdot}CO(OPO_3H_2) \rightleftharpoons CH_3{\cdot}CO{\cdot}C^*OOH + H_3PO_4$

$(C^*O_2 + 2H \rightarrow HC^*OOH)$

Fig. 3.—Bacterial fixation of CO_2.

In 1941, Krampitz and Werkman[21] separated an enzyme preparation (oxalacetate β-carboxylase) from *Micrococcus lysodeikticus* which decarboxylates oxalacetic acid to form pyruvic acid. Cocarboxylase was not required for this reaction. When oxalacetate, the enzyme and magnesium ions were incubated in the presence of $C^{13}O_2$ until approximately half of the oxalacetate disappeared, C^{13} was found in the remaining oxalacetate.[22] The isotope was found exclusively in the carboxyl group adjacent to the methylene group. Since the reaction medium contained only oxalacetate and CO_2, and since the only products formed were pyruvic acid and CO_2, it was concluded that this reaction was reversible.

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(18) H. G. Wood and C. H. Werkman, *J. Bact.*, **30**, 332 (1935).

(19) H. G. Wood and C. H. Werkman, *Biochem. J.*, **30**, 48 (1936).

(20) H. G. Wood, R. W. Stone and C. H. Werkman, *Biochem. J.*, **31**, 349 (1937).

(21) L. O. Krampitz and C. H. Werkman, *Biochem. J.*, **35**, 595 (1941).

(22) L. O. Krampitz, H. G. Wood and C. H. Werkman, *J. Biol. Chem.*, **147**, 243 (1943).

The subsequent conversion of oxalacetate to succinate is believed to occur by way of malate and fumarate since Krebs and Eggleston[23] have demonstrated that the following reactions occur reversibly in propionic acid bacteria.

$$HOOC\cdot CH_2\cdot CO\cdot COOH \rightleftharpoons HOOC\cdot CH_2\cdot CHOH\cdot COOH \rightleftharpoons HOOC\cdot CH{:}CH\cdot COOH \rightleftharpoons HOOC\cdot CH_2\cdot CH_2\cdot COOH$$

In accordance with these reactions it has been found that isotopic CO_2 is assimilated by propionic acid bacteria to produce among other products, succinic acid containing isotopic carbon localized in the carboxyl groups.[24] In general, it has been observed that those organisms which form succinic, propionic, or lactic acids are able to fix isotopic CO_2 in the carboxyl positions.

That CO_2 fixation may involve other pathways is suggested by the finding that several microorganisms can convert isotopic CO_2 to carboxyl-labelled acetic acid.[25,7]

Slade and Werkman[26] suggest that the resulting acetate may be formed by incorporation of CO_2 into oxalacetate, then into succinate followed by reductive cleavage.

$$HOOC^{13}\cdot CH_2\cdot CH_2\cdot C^{13}OOH + 2H \rightleftharpoons 2CH_3\cdot C^{13}OOH$$

These investigators have shown that suspensions of *Aerobacter indologenes* convert carboxyl-labelled acetate into isotopic succinate and *vice versa.* In both cases the isotope remained fixed in the carboxyl groups. Furthermore, doubly labelled acetate is converted to succinic acid which has the isotope equally distributed between the carboxyl and methylene groups. This is strong evidence that acetic acid can undergo condensation to form succinic acid without participation of carbon dioxide as an intermediate.

Barker and Kamen[7] have recently shown that acetate labelled in both positions is obtained when *Clostridium thermoaceticum* assimilates $C^{14}O_2$ during the fermentation of D-glucose. The reactions involved are shown in Fig. 3, reaction II B. The D-glucose was quantitatively converted to acetate. The CO_2 resulting from the reaction is believed to be reductively condensed to acetate. Wieringa[27] has furnished supporting eivdence by demonstrating that another species of *Clostridium* can

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(23) H. A. Krebs and L. V. Eggleston, *Biochem. J.*, **35**, 676 (1941).

(24) H. G. Wood, C. H. Werkman, A. Hemingway and A. O. Nier, *J. Biol. Chem.*, **139**, 377 (1941).

(25) H. D. Slade, H. G. Wood, A. O. Nier, A. Hemingway and C. H. Werkman, *J. Biol. Chem.*, **143**, 133 (1942).

(26) H. D. Slade and C. H. Werkman, *Arch. Biochem.*, **2**, 97 (1943).

(27) K. T. Wieringa, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **3**, 1 (1936); **6**, 251 (1940).

reduce CO_2 to acetate in the presence of hydrogen. Barker, Kamen and Haas[28] have also shown that *Butyribacterium rettgeri* can convert $C^{14}O_2$ to doubly labelled acetic acid (and also to completely labelled butyric acid) during lactic acid fermentation. This reaction has also been found to occur in *Cl. acidi-urici* (Fig. 3, reaction II C).

Lipmann's studies[29] on pyruvate oxidation by *Lactobacillus delbrückii* have suggested the existence of another type of carbon-carbon condensation. Excellent evidence was obtained indicating that acetyl phosphate is an important intermediate in these metabolic reactions and a reversible reaction between acetyl phosphate and formate to form pyruvate and inorganic phosphate was postulated (see Fig. 3). Utter, Lipmann and Werkman[30] were able to demonstrate the reversibility of this reaction with formate containing C^{13}. After incubating isotopic formate and non-isotopic pyruvate with extracts of *Escherichia coli* both substances were isolated and found to contain the same concentration of C^{13} in their respective carboxyl groups. Thus the excess C^{13} which was introduced as formate was in equilibrium with the carboxyl group of pyruvate. CO_2 was excluded as an intermediate in this reaction since the CO_2 in the system contained only a very small amount of C^{13}. The pyruvate carboxyl could not, therefore, have acquired its isotope by the reaction of CO_2 with pyruvate to form oxalacetate followed by subsequent decarboxylation. Intact *E. coli* can, however, reduce CO_2 to formate, with hydrogen.[31]

Koepsell and Johnson,[32] and Koepsell, Johnson and Meek[33] have reported a similar reaction obtained with an extract prepared from *Clostridium butylicum*. In this system pyruvate is converted to acetate, CO_2 and hydrogen (Fig. 3). Formate is not oxidized by this enzyme system. In addition to supporting evidence by Lipmann and Tuttle,[16] Wilson, Krampitz and Werkman[34] have established the validity of this reaction with $C^{13}O_2$. The isotopic CO_2 was found to exchange rapidly with the carboxyl group of pyruvate in the same system.

Although the evidence is quite convincing that microorganisms can assimilate CO_2 by condensation with a two-carbon compound (which is probably acetyl phosphate), it is not known whether acetyl phosphate is

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(28) H. A. Barker, M. D. Kamen and V. Haas, *Proc. Natl. Acad. Sci. U. S.*, **31**, 355 (1945).

(29) F. Lipmann, *Cold Springs Harbor Symposia Quant. Biol.*, **7**, 248 (1939).

(30) M. F. Utter, F. Lipmann and C. H. Werkman, *J. Biol. Chem.*, **158**, 521 (1945).

(31) D. D. Woods, *Biochem. J.*, **30**, 515 (1936).

(32) H. J. Koepsell and M. J. Johnson, *J. Biol. Chem.*, **145**, 379 (1942).

(33) H. J. Koepsell, M. J. Johnson and J. S. Meek, *J. Biol. Chem.*, **154**, 535 (1944).

(34) J. Wilson, L. O. Krampitz and C. H. Werkman, Private communication quoted by H. G. Wood, *Physiol. Revs.*, **26**, 198 (1946).

present in or plays a similar metabolic role in animal tissue. The pyruvate-CO_2 condensation (Wood-Werkman reaction) forming oxalacetate has, however, been convincingly established as an important metabolic reaction in animals.

The role of CO_2 in metabolism has likewise been studied with unicellular organisms other than bacteria. Foster and coworkers[35] have demonstrated that the molds *Rhizopus nigricans* and *Aspergillus niger* can ferment D-glucose and sucrose in a medium containing $C^{11}O_2$ to form isotopic fumaric and citric acids, respectively. The C^{11} was found to be present only in the carboxyl groups. The protozoan *Tetrahymena geleii* assimilates $C^{11}O_2$, fixing it in the carboxyl groups of succinic acid.

VI. The Tricarboxylic Cycle and Carbon Dioxide Assimilation in Animal Tissues

The oxidation of carbohydrates by animal tissue is currently believed to proceed mainly by way of the so called "tricarboxylic acid cycle."

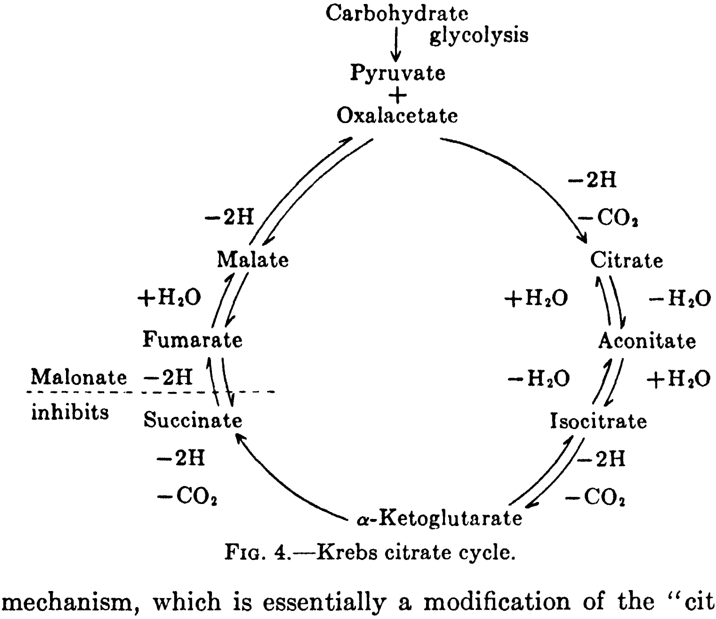

FIG. 4.—Krebs citrate cycle.

This mechanism, which is essentially a modification of the "citric acid cycle" originally proposed by Krebs and Johnson,[36] appears to explain best the experimental findings even though complete proof supporting every step of the cycle has not as yet been obtained (Fig. 4).

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(35) J. W. Foster, S. F. Carson, S. Ruben and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **27**, 590 (1941).

(36) H. A. Krebs and W. A. Johnson, *Enzymologia*, **4**, 148 (1937).

The early work of Szent-Györgyi[37] as well as Stare and Baumann[38] had indicated that oxidation (oxygen consumption) in isolated tissues was stimulated by the addition of catalytic amounts of dicarboxylic acids such as succinic, fumaric, malic and oxalacetic. Accordingly these dicarboxylic acids were considered to play an important part in the hydrogen transport systems involved in tissue oxidations. Krebs and Eggleston[39] demonstrated that oxidation of pyruvate in pigeon muscle could be accelerated by the addition of catalytic amounts of such dicarboxylic acids. Since very small amounts of oxalacetic acid could effect the oxidation of large quantities of pyruvate it appeared probable that oxalacetic acid was regenerated by the cyclic reactions involved in the oxidation of pyruvate (Fig. 4).

The addition of malonate, a specific inhibitor of the succinic dehydrogenase system, inhibits the regeneration of oxalacetic acid and results in the accumulation of citric, α-ketoglutaric and succinic acids. Upon exhaustion of the remaining stores of oxalacetic acid, pyruvate oxidation in muscle ceases and can only be resumed after further addition of oxalacetic acid or addition of any dicarboxylic acid capable of conversion to oxalacetic acid. Under these conditions, the amount of pyruvate oxidized is equivalent to the amount of oxalacetate supplied.

In pigeon liver, however, this effect is not observed. Evans[40] found that pigeon liver mince, poisoned with malonate, does not require the addition of dicarboxylic acids for pyruvate oxidation. This tissue is apparently capable of synthesizing its own dicarboxylic acids. Krebs and Eggleston[41] working with normal pigeon liver mince observed that exclusion of bicarbonate from the medium resulted in decreased oxidation of pyruvate and, hence, decreased production of dicarboxylic acids. They accordingly suggested that dicarboxylic acids were produced from pyruvate and CO_2 by means of the Wood-Werkman reaction (see Fig. 3, Equation I) and that the resulting oxalacetate condensed with pyruvate (or its equivalent) to form citrate (see Fig. 4).

In the same year, Ruben and Kamen[42] obtained direct evidence for this hypothesis by demonstrating fixation of $C^{11}O_2$ in rat liver. Furthermore, Evans and Slotin[43] successfully isolated radioactive α-ketoglutaric acid from malonate-poisoned pigeon liver mince which had been incu-

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(37) A. Szent-Györgyi, *Z. physiol. Chem.*, **235**, 1 (1935); **244**, 105 (1936).

(38) F. J. Stare and C. A. Baumann, *Proc. Roy. Soc. London*, **B121**, 338 (1936).

(39) H. A. Krebs and L. V. Eggleston, *Biochem. J.*, **34**, 442 (1940).

(40) E. A. Evans, Jr., *Biochem. J.*, **34**, 829 (1940).

(41) H. A. Krebs and L. V. Eggleston, *Biochem. J.*, **34**, 1383 (1940).

(42) S. Ruben and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.*, **26**, 418 (1940).

(43) E. A. Evans, Jr., and L. Slotin, *J. Biol. Chem.*, **136**, 301 (1940); **141**, 439 (1941).

bated with pyruvate and $NaHC^{11}O_3$. These workers, and simultaneously Wood and coworkers,[44] demonstrated that the isolated α-ketoglutaric acid contained isotope primarily in the carboxyl group α to the carbonyl (Fig. 5).

This finding ruled out citrate as a direct intermediate in the tricarboxylic acid cycle since isotopic citric acid, which is a symmetrical compound, could only give rise to α-ketoglutaric acid containing isotope distributed equally in both carboxyl groups (Fig. 5). Addition of citrate to their system (Evans and Slotin), furthermore, did not affect the specific radioactivity of the α-ketoglutarate. This effect would have been obtained had citrate been an intermediate.

Further proof that CO_2 is assimilated by means of the enzyme oxalacetate β-carboxylase was obtained by Evans and coworkers,[45] who succeeded in preparing a cell-free preparation of this enzyme from liver. The enzyme was able to catalyze the decarboxylation of oxalacetate to pyruvate. These investigators were able to demonstrate an uptake of $C^{11}O_2$. Utter and Wood[46] have demonstrated conclusively, however, that in the presence of isotopic CO_2, pyruvate can be converted to oxalacetate containing isotopic carbon and that the process is, of course, reversible. Addition of adenosine triphosphate to this liver enzyme system increased the rate of incorporation of $C^{13}O_2$. Wood, Vennesland and Evans[47] have also shown that during the fixation of CO_2, isotopic carbon is incorporated solely and in equal concentrations into the carboxyl groups of pyruvate, lactate, malate and fumarate.

Wood and coworkers[44] have demonstrated that pigeon liver mince poisoned with malonate is unable to convert $C^{13}O_2$ into isotopic succinate but can direct the isotope into malate and fumarate as well as into the α-carboxyl of α-ketoglutarate (see Fig. 5). On the other hand, non-poisoned liver possesses the ability to anaerobically fix $C^{13}O_2$ in succinic acid as well as fumaric and malic acids. The dicarboxylic acids invariably contain the isotopic carbon predominantly in their respective carboxyl groups. These important experiments provide conclusive proof for two main concepts. First, that malonate specifically inhibits succinic acid dehydrogenase and hence blocks completely the formation of succinic from fumaric acid. Secondly, there must be two routes by which succinate is formed. By the first pathway, pyruvate can condense with

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(44) H. G. Wood, C. H. Werkman, A. Hemingway and A. O. Nier, *J. Biol. Chem.*, **139**, 483 (1941); **142**, 31 (1942).

(45) E. A. Evans, Jr., B. Vennesland and L. Slotin, *J. Biol. Chem.*, **147**, 771 (1943).

(46) M. F. Utter and H. G. Wood, *J. Biol. Chem.*, **160**, 375 (1945).

(47) H. G. Wood, B. Vennesland and E. A. Evans, Jr., *J. Biol. Chem.*, **159**, 153 (1945).

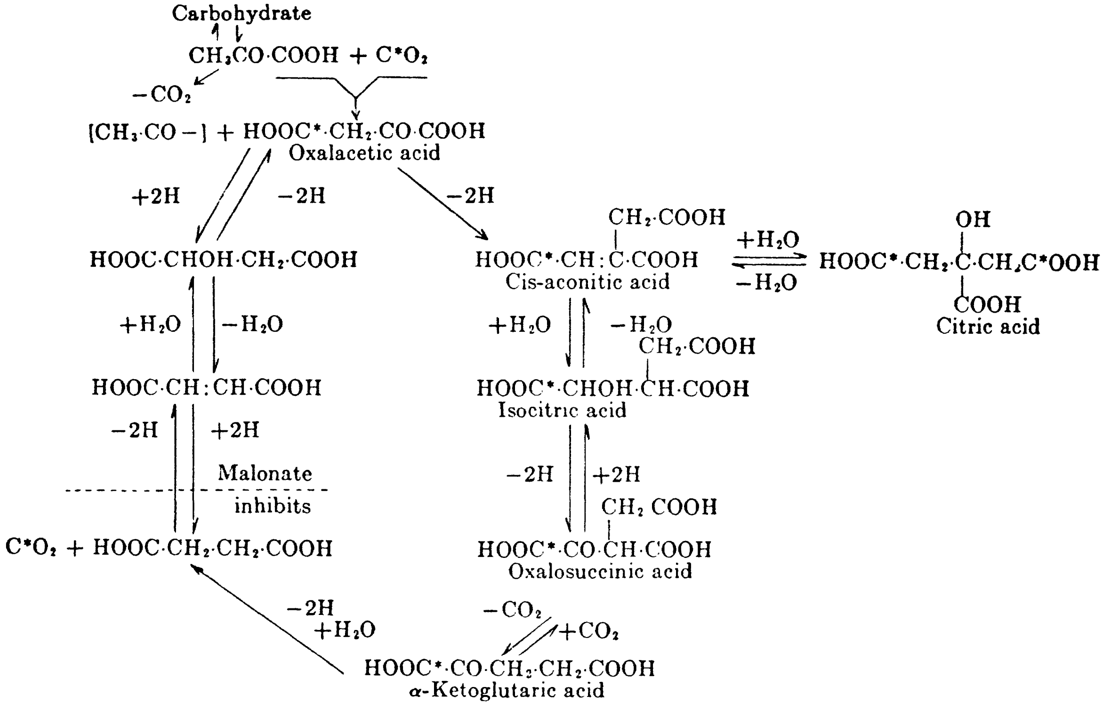

FIG. 5.—Tricarboxylic acid cycle.

CO_2 to form oxalacetate which under anaerobic conditions is reduced to malate. The malate in turn may be converted to fumarate and succinate (Fig. 5). The last step in this series of reactions is blocked by malonate. The second pathway involves the aerobic condensation of pyruvate and oxalacetate followed by oxidation of the condensation product to form α-ketoglutarate and succinate. Wood[48] has proposed that the first condensation product of the aerobic tricarboxylic cycle is *cis*-aconitic acid which is then converted to succinate by way of isocitric, oxalosuccinic, and α-ketoglutaric acids. The α-ketoglutarate is decarboxylated and oxidized to succinic acid. Isotopic α-ketoglutarate containing isotopic carbon only in the carboxyl group located α to the carbonyl would be expected to yield non-isotopic succinate after decarboxylation. This accounts for the absence of isotopic carbon in succinate isolated from malonate-poisoned liver after incubation with pyruvate and isotopic bicarbonate.

Although citrate has been excluded as the primary condensation product of pyruvate and oxalacetate, no direct evidence bearing upon the nature of this product has as yet been obtained. The participation of *cis*-aconitic and isocitric acids is speculative. Nor is there any evidence supporting the hypothesis that pyruvate and oxalacetate condense to form a hypothetical intermediate oxalcitraconic acid[49] which can be oxidatively decarboxylated to citric acid. Since citrate, aconitate and isocitrate are in equilibrium with each other, the participation of the last two substances as intermediates of carbohydrate oxidation would, on the surface, appear to be doubtful. Krebs,[49] however, believes that the conversion of *cis*-aconitate to α-ketoglutarate occurs so rapidly in liver that equilibrium with citrate is not attained.

Wood[48] has recently proposed a condensation of oxalacetate with a two-carbon intermediate as the initial reaction. This proposal, which has also been suggested by Krebs,[49] and Martius,[50] states that pyruvate may be oxidatively decarboxylated to a two-carbon intermediate (not necessarily acetate) which can then condense with oxalacetate to form *cis*-aconitate (Fig. 5). Although acetyl phosphate along with other possible two-carbon intermediates has been suggested, no convincing evidence for its participation has as yet been obtained. It is of interest, however, that Buchanan and coworkers[51] have demonstrated by means of C^{13} that kidney homogenate oxidizes isotopic acetate and acetoacetate

(48) H. G. Wood, *Physiol. Revs.*, **26**, 198 (1946).

(49) H. A. Krebs, *Advances in Enzymology*, **3**, 191 (1943).

(50) K. Martius, *Z. physiol. Chem.*, **279**, 96 (1943).

(51) J. M. Buchanan, W. Sakami, S. Gurin and D. W. Wilson, *J. Biol. Chem.*, **159**, 695 (1945).

by a pathway common to carbohydrate. The isotopic carbon of these substances finds its way into α-ketoglutarate, fumarate and succinate. In this series of reactions, citrate was again excluded as a possible intermediate. Furthermore, it was proposed that a metabolically active two-carbon substance arising from acetate or acetoacetate could condense with oxalacetate to form *cis*-aconitate or isocitrate.

Ochoa[52] has recently reported a new assimilation reaction involving α-ketoglutarate and CO_2 to form oxalosuccinic acid. This provides another pathway by which CO_2 may be utilized. The mechanisms involved in this new assimilation reaction are thoroughly discussed by Ochoa.[52a]

Although the tricarboxylic acid cycle satisfactorily explains the oxidation of pyruvate to carbon dioxide and water in isolated tissues, further evidence is needed to clarify a number of steps of the cycle and to evaluate its full significance in the intact animal.

VII. Carbohydrate Synthesis in Animal Tissues

Balance studies carried out with fasted, phlorizinized, depancreatized, and other types of "experimentally diabetic" animals have yielded a great deal of information concerning those substances which are "sugar formers" and those which are not. All substances involved as intermediates in glycolysis or in the tricarboxylic acid cycle, along with metabolites which can be converted into compounds which increase the amounts of these substances, are capable of forming "extra" D-glucose or glycogen. On the other hand, intermediates of fat oxidation are considered to be non-sugar forming.

Carbohydrate precursors containing C^{11} or C^{13} have been synthesized and administered to fasting or to phlorizinized rats. In the course of such experiments with fasted rats, Solomon and coworkers[53] fed non-isotopic lactic acid and simultaneously injected radioactive sodium bicarbonate. Because of the rapidity with which liver glycogen is regenerated under these conditions, an appreciable amount of liver glycogen was isolated and found to be radioactive. These results convincingly established the fixation of CO_2 by normal animals. The glycogen contained 0.3–1.5% of the administered C^{11} and it was calculated that approximately 11% of the liver glycogen was derived from CO_2. Similar results were obtained when D-glucose was used instead of lactate. Lac-

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(52) S. Ochoa, *J. Biol. Chem.*, **159**, 243 (1945).

(52*a*) S. Ochoa *in* Green, D. E., "Currents in Biochemical Research," p. 165, Interscience Publishers, N. Y. (1946).

(53) A. K. Solomon, B. Vennesland, F. W. Klemperer, J. M. Buchanan and A. B. Hastings, *J. Biol. Chem.*, **140**, 171 (1941).

tate labelled in either the α or β positions was likewise converted into glycogen with approximately 16% of the resulting glycogen apparently derived from lactate. In the case of carboxyl-labelled lactate[54,55] only 6% of the glycogen formed was derived from the lactate. Buchanan and coworkers[56] have likewise demonstrated that carboxyl-labelled propionate and butyrate can similarly give rise to radioactive glycogen. Although their experiments failed to demonstrate conversion of acetate carbon to glycogen, Rittenberg and Bloch[57] succeeded in proving that administration of carboxyl-labelled acetate to rats results in the incorporation of C^{13} in liver glycogen.

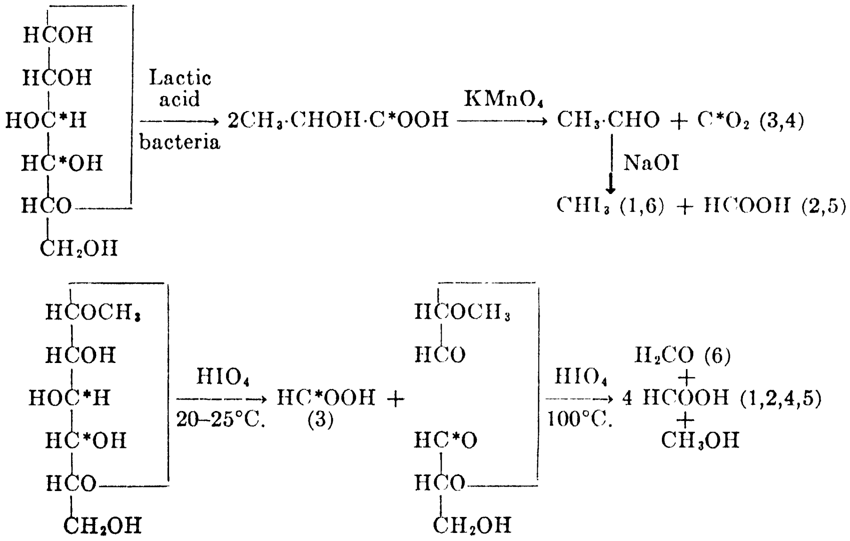

FIG. 6.—Chemical degradation of isotopic D-glucose.

Wood and coworkers[58] have repeated this work with C^{13}. When isotopic $NaHC^{13}O_3$ was administered, the liver glycogen was found to contain excess C^{13}. After conversion of the isotopic glycogen to D-glucose, degradation of the resulting D-glucose indicated that carbons 3 or 4 were isotopic (Fig. 6). Furthermore, the concentration of C^{13} was the same in both carbons. Lorber and coworkers[59] carried out the same type

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(54) J. B. Conant, R. D. Cramer, A. B. Hastings, F. W. Klemperer, A. K. Solomon and B. Vennesland, *J. Biol. Chem.*, **137,** 557 (1941).

(55) R. D. Cramer and G. B. Kistiakowsky, *J. Biol. Chem.*, **137,** 549 (1941).

(56) J. M. Buchanan, A. B. Hastings and F. B. Nesbett, *J. Biol. Chem.*, **150,** 413 (1943).

(57) D. Rittenberg and K. Bloch, *J. Biol. Chem.*, **154,** 311 (1944).

(58) H. G. Wood, N. Lifson and V. Lorber, *J. Biol. Chem.*, **159,** 475 (1945).

(59) V. Lorber, N. Lifson and H. G. Wood, *J. Biol. Chem.*, **161,** 411 (1945).

of experiment with $CH_3{\cdot}C^{13}OOH$, $C^{13}H_3{\cdot}C^{13}OOH$, $CH_3{\cdot}CH_2{\cdot}C^{13}OOH$ and $CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}C^{13}OOH$. In all cases, the recovered glycogen contained an excess of C^{13}. Furthermore, all of the acids labelled in the carboxyl group transferred their isotope to carbons 3 or 4 of the resulting D-glucose. With acetate labelled in both carbons, however, all of the carbons of D-glucose were found to contain C^{13}.

Since the carbon of carbon dioxide is assimilated into positions three and four of D-glucose, it is always necessary to correct for the isotopic carbon which finds its way into metabolic CO_2 when biological conversion to carbohydrate is studied. The results obtained with doubly labelled acetate prove conclusively that a part of the acetate molecule can be converted to glycogen or D-glucose without first forming CO_2. Buchanan and coworkers,[56] after making suitable corrections for incorporation of CO_2, have concluded that carboxyl-labelled propionate and butyrate form glycogen to a significant extent. Lorber, Hemingway and Nier[60] have also demonstrated the incorporation of $C^{13}O_2$ into muscle glycogen.

The fixation of CO_2 in carbohydrate must be reconciled with two factors: (1) the C^*O_2 must in some way enter the carboxyl group of pyruvic acid since the isotope is eventually found exclusively in carbons 3 or 4 of the glycogen; (2) the formation of phosphopyruvate prior to the synthesis of D-glucose is an essential step. This conversion of pyruvate to phosphopyruvate was considered to be irreversible until Lardy and Ziegler[61] demonstrated that this reaction can occur in extracts of rat muscle in the presence of potassium ions.

It is at present believed that the incorporation of isotopic CO_2 into the carboxyl group of pyruvic acid occurs by the following pathway.

$$\begin{tikzcd}
\mathrm{C^*O_2 + CH_3{\cdot}CO{\cdot}COOH} \arrow[r, phantom, "\rightleftharpoons"] \arrow[d, "\text{or}"] & \mathrm{HOOC^*{\cdot}CH_2{\cdot}CO{\cdot}COOH} \arrow[d, shift left] \\
\mathrm{HOOC^*{\cdot}CH{:}CH{\cdot}C^*OOH} \arrow[r, phantom, "\rightleftharpoons"] \arrow[d, shift left] & \mathrm{HOOC^*{\cdot}CH_2{\cdot}CHOH{\cdot}COOH} \arrow[u, shift left] \\
\mathrm{HOOC^*{\cdot}CH_2{\cdot}CHOH{\cdot}C^*OOH} \arrow[u, shift left] \arrow[r, phantom, "\rightleftharpoons"] & \mathrm{HOOC^*{\cdot}CH_2{\cdot}CO{\cdot}C^*OOH} \arrow[r, phantom, "\rightleftharpoons"] & \mathrm{CH_3{\cdot}CO{\cdot}C^*OOH}
\end{tikzcd}$$

$$\mathrm{CH_3{\cdot}CO{\cdot}C^*OOH} + \text{adenosine triphosphate} \rightarrow \mathrm{CH_2{:}C(OPO_3H_2){\cdot}C^*OOH} + \text{adenosine diphosphate}$$

Although it had been proposed[45] that the enolic form of oxalacetate undergoes a shift of its enolic group thus giving rise to carboxyl-labelled pyruvate, this mechanism has been disproved by Krampitz and coworkers.[22]

It is possible, however, that phosphopyruvate is formed in another way. Kalckar[62] has suggested that phosphopyruvate synthesis occurs

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(60) V. Lorber, A. Hemingway and A. O. Nier, *J. Biol. Chem.*, **151**, 647 (1943).

(61) H. A. Lardy and J. A. Ziegler, *J. Biol. Chem.*, **159**, 475 (1945).

(62) H. M. Kalckar, *Biochem. J.*, **33**, 631 (1939).

during fumarate oxidation, and Lipmann[63] proposed the addition of inorganic phosphate to fumarate followed by oxidation to phospho-oxalacetate. This compound could then be decarboxylated to phosphopyruvate (Fig. 7).

In this way Solomon and coworkers[53] attempted to explain the conversion of lactate, pyruvate and carbon dioxide to glycogen. According to the proposed mechanism, carboxyl-labelled pyruvate must first undergo the Wood-Werkman reaction to form oxalacetate before carboxyl-labelled phosphopyruvate can be formed by way of fumarate, phosphomalate and phospho-oxalacetate. This hypothesis is no longer necessary in view of Lardy and Ziegler's results described above. It is possible, however, that both pathways are utilized.

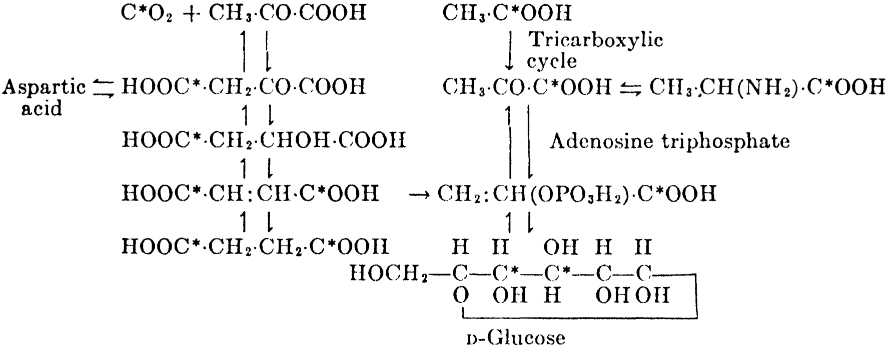

FIG. 7.—Biological synthesis of D-glucose and glycogen.

Boxer and Stetten[3] have demonstrated that the administration to fasted rats of heavy water along with non-isotopic lactate leads to the formation of deuterium-labelled liver and muscle glycogen. The deuterium was distributed in practically all the positions of the glycogen molecule. In a similar type of experiment, non-isotopic D-glucose administered along with heavy water resulted in the incorporation of considerably less deuterium in the glycogen. These authors believe that glycogen containing deuterium is synthesized from small fragments (trioses) which can exchange their hydrogen for deuterium whereas the direct conversion of D-glucose to glycogen involves incorporation of very little deuterium. From their experiments they calculate that although the well-fed rat converts only 3% of the D-glucose to glycogen, 30% of the D-glucose supplement is actually transformed into fatty acids.

The conversion of glycine to glycogen by the rat has been studied by Olsen, Hemingway and Nier[64] who have demonstrated that glycine

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(63) F. Lipmann, *Advances in Enzymology*, **1**, 99 (1941).

(64) N. S. Olsen, A. Hemingway and A. O. Nier, *J. Biol. Chem.*, **148**, 611 (1943).

labelled with C^{13} in the carboxyl group is utilized to a small extent in the synthesis of isotopic glycogen.

VIII. THE INTERRELATIONSHIP OF CARBOHYDRATE AND FATTY ACID METABOLISM*

Although D,L-alanine is a "sugar former" in the "diabetic" animal, nevertheless, the administration of carboxyl-labelled D,L-alanine to the fasted phlorizinized rat results in the excretion of D-glucose containing only a few per cent of the isotopic carbon administered.[1] Apparently much of the alanine is diverted to other metabolic pathways. In contrast to these results, the administration of lactate labelled with C^{14} in the α or β positions resulted in the excretion of urinary D-glucose containing approximately 25% of the administered isotope.[65] Furthermore, in confirmation of Stetten and Boxer's results,[2] some 40% of the administered isotope was recovered in the fat of the animal. It was also demonstrated for the first time in the intact animal that the carbon of lactate was converted directly to ketone bodies. The urinary acetoacetate and β-hydroxybutyrate obtained after the administration of α,β-labelled lactate to the phlorizinized rat contained isotopic carbon distributed equally among all four carbon atoms. These results are not surprising in view of the extensive literature indicating that liver slices[66,67,68,69] and homogenates[70] are capable of transforming pyruvate into acetoacetate. Washed homogenates of liver are able to convert isotopic pyruvate labelled in the α or β positions to completely labelled acetoacetate.[71] The over-all reactions may be pictured as follows:

$$2C^*H_3{\cdot}C^*HOH{\cdot}COOH \rightarrow 2C^*H_3{\cdot}C^*O{\cdot}COOH \rightarrow C^*H_3C^*O{\cdot}C^*H_2{\cdot}C^*OOH + 2CO_2$$

It is apparent, therefore, that there are some metabolic pathways common to the biological utilization of both cabohydrate and fat.

That fatty acids may be oxidized by way of the tricarboxylic cycle has received serious consideration in recent years. Breusch[72] has suggested that β-keto acids resulting from the biological oxidation of long-chain fatty acids are able to condense with oxalacetate under the influence of an enzyme (citrogenase) to form citrate. This enzyme is believed to be specific for a number of β-keto acids.

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* For a general discussion, see H. J. Deuel and Margaret G. Morehouse, *Advances in Carbohydrate Chem.*, **2**, 119 (1946).

(65) D. W. Wilson, A. Delluva and S. Gurin, *Proc. Am. Soc. Exptl. Biol.*, **6**, 302 (1947).

(66) E. Annau, *Z. physiol. Chem.*, **224**, 141 (1934).

(67) N. L. Edson, *Biochem. J.*, **29**, 2082 (1935).

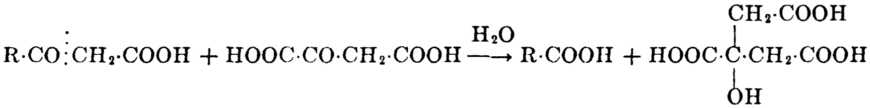
(68) H. Weil-Malherbe, *J. Soc. Chem. Ind.*, **55**, 838 (1936).

(69) H. A. Krebs and W. A. Johnson, *Biochem. J.*, **31**, 645 (1937).

(70) A. L. Lehninger, *J. Biol. Chem.*, **161**, 413 (1945).

(71) D. I. Crandall, S. Gurin and D. W. Wilson, *Proc. Am. Soc. Exptl. Biol.*, **6**, 246 (1947).

(72) F. L. Breusch, *Science*, **97**, 490 (1943).



$$\mathrm{R\cdot CO\vdots CH_2\cdot COOH + HOOC\cdot CO\cdot CH_2\cdot COOH \xrightarrow{H_2O} R\cdot COOH + HOOC\cdot \overset{CH_2\cdot COOH}{\underset{OH}{C}}\cdot CH_2\cdot COOH}$$

The resulting products are citric acid and a fatty acid reduced in length by two carbons. It is postulated that the remaining aliphatic acid after subsequent oxidation to the corresponding β-keto acid undergoes a similar condensation. In this manner the complete breakdown of fatty acid chains is explained.

Whether or not this theory is correct, it is true that acetate and acetoacetate can condense with oxalacetate to form citrate.[73]

Lynen,[74] and Virtanen and Sundman[75] have reported that in yeast, acetate is metabolized by way of the tricarboxylic cycle. Wieland and Rosenthal[73] have also shown that kidney contains an enzyme system which can convert acetate or acetoacetate to citrate. Upon addition of oxalacetate along with either of these acids, the yield of citrate is significantly increased. In these experiments barium ions were added to inhibit further breakdown of the resulting citrate. These authors have proposed that oxalacetate and acetoacetate condense to form either acetyl citrate or citroyl acetate which are subsequently hydrolyzed to citrate and acetate.

Although several theories have been proposed to explain the formation of citrate, the only fact that rests on firm experimental ground is that acetate, pyruvate, acetoacetate and some β-keto acids can, under proper experimental conditions, condense with oxalacetate to form citrate.

In this connection it is of interest that Buchanan and coworkers[51] demonstrated that isotopic acetoacetate ($CH_3\cdot C^{13}O\cdot CH_2\cdot C^{13}OOH$) and acetate ($CH_3\cdot C^{*}OOH$) are oxidized in kidney by way of the tricarboxylic cycle. In several experiments of this type, α-ketoglutarate, fumarate and succinate were isolated after incubation with isotopic acetoacetate or acetate. The isolated acids were found to contain sufficient excess of isotope to warrant the belief that acetoacetate and acetate are oxidized in kidney to a major extent by way of the tricarboxylic cycle. A similar result with carboxyl-labelled acetate has been obtained by Weinhouse and coworkers.[76]

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(73) H. Wieland and C. Rosenthal, *Ann.*, **554**, 241 (1945).

(74) F. Lynen, *Ann.*, **552**, 270 (1942); **554**, 40 (1945).

(75) A. J. Virtanen and S. Sundman, *Biochem. Z.*, **313**, 236 (1942).

(76) S. Weinhouse, G. Medes, N. F. Floyd and L. Noda, *J. Biol. Chem.*, **161**, 745 (1945).

A pathway by which acetate and acetoacetate are oxidized is indicated in Fig. 8. The similarity to the mechanism of oxidation of pyruvate is striking. According to this proposed scheme acetate and acetoacetate are converted to a hypothetical two-carbon intermediate which is capable of condensing with oxalacetate or one of the other four-carbon dicarboxylic acids to yield *cis*-aconitate or isocitrate. The rest of the cycle is identical with the carbohydrate tricarboxylic cycle. Reference to this cycle illustrates several points of interest. It makes clear the pathway by which labelled carbon in acetate or acetoacetate may be transformed into D-glucose or glycogen. Since oxalacetate labelled in either carboxyl position is generated in the cycle, carboxyl-labelled pyruvic acid may be readily formed as a result of decarboxylation. The resulting pyruvic acid can then be phosphorylated directly. If phosphopyruvate is produced by phosphorylation of fumarate *via* phosphomalate and phospho-oxalacetate (Fig. 7), it is evident that phosphopyruvate labelled in the carboxyl position may be formed by either mechanism. From this substance isotopic D-glucose or glycogen can be synthesized. Isotopic carbon dioxide, as well as carboxyl-labelled lactate, pyruvate and acetate will all yield carboxyl-labelled phosphopyruvate and, therefore, D-glucose and glycogen labelled predominantly in the 3,4 positions (Fig. 7).

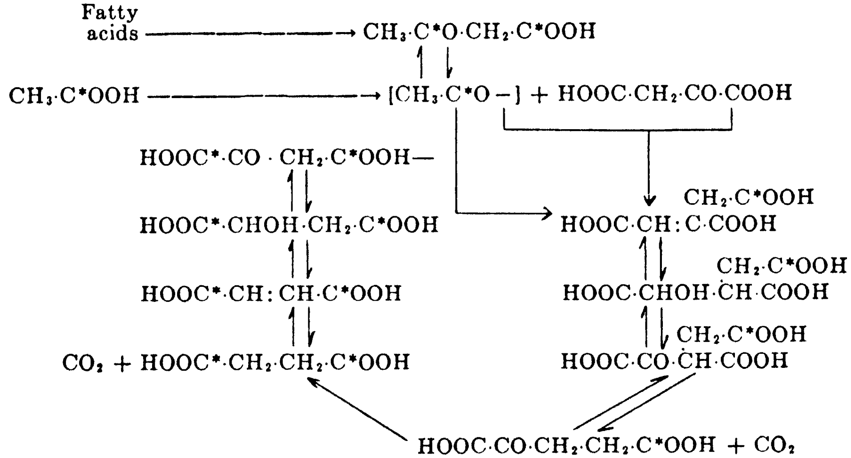

FIG. 8.—Oxidation of acetoacetate, acetate and fatty acids by the tricarboxylic acid cycle.

This scheme (Fig. 8) also explains how acetic, acetoacetic, and fatty acids may be oxidized by a mechanism common to carbohydrate oxidation without effecting a net synthesis of carbohydrate. The over-all reaction of this cycle is:

$$CH_3{\cdot}COOH \rightarrow 2CO_2 + 2H_2O.$$

The carbons of the acetic acid, however, are not the ones which have formed carbon dioxide. Thus, acetic acid carbon atoms may be found in carbohydrate precursors without causing the accumulation of carbohydrate.

It is apparent that the metabolic pathways of carbohydrate and fat as well as protein are interwoven in a most complex fashion. Over-all balance studies are unable to unravel this maze of interlocking reactions. The isotopes, on the other hand, furnish a tool which will be of the utmost value in the study of these problems.

PRODUCTS OF THE ENZYMIC DEGRADATION OF STARCH AND GLYCOGEN[1]

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(1) In this paper no attempt is made to review the rich literature on starch and its enzymic degradation. On the contrary the author has only attempted to present his

I. Constitution of Starch and Glycogen

Completely methylated starch yields on acid hydrolysis more than 90% 2,3,6-trimethyl-D-glucose. This proves that more than 90% of the D-glucosidic linkages of the polysaccharide are 1,4-linkages. The investigations and calculations of Freudenberg[2] show that all (or almost all) D-glucosidic linkages in the starch must be α-D-glucosidic. Gentiobiose, which has been isolated in small amounts from starch conversion liquors,[3] is probably formed by "reversion" from D-glucose.[4]

The end group assay, as developed chiefly by Haworth and coworkers, shows starch to contain about 4.5% end groups. This has been confirmed by many investigators. It is possible that starches of different origins have somewhat different end group contents, but the differences can only be small. From these results, Haworth concluded that the starch molecule is a simple chain of about 25 D-glucose residues united by the 1,4-α-D-glucosidic link (Fig. 1, formula III). (In the formulas given in this paper the D-glucose residue is represented by an arrow, the arrow head denoting the reducing group and the tail the position 4. Two arrows in the head to tail combination thus indicate maltose, formula I. The position 6 is represented by the middle of the arrow shaft. Isomaltose, 6-α-D-glucosyl-D-glucose, has the formula II.)

The conception of the starch molecules as chains of D-glucose residues united with 1,4-α-D-glucosidic linkages (maltose linkages) is in accordance with the fact, known long ago,[5] that the main product of the enzymic degradation is always maltose, provided that no secondary scission to D-glucose by action of maltase occurs. But it is just as well known that the conversion to maltose is seldom or never complete. Generally, the yield of maltose is about 70%, the remainder consisting of saccharides with higher molecular weight, that is, with a *degree of polymerization* (*D.P.*) > 2. These saccharides, the *limit dextrins*, are attacked by the amylases either not at all or only extremely slowly, and therefore the starch conversion generally reaches a more or less definite *limit of saccharification*. Different explanations have been given.

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own interpretation of the connection between the chemical constitution of starch and known facts concerning the breakdown and the products formed thereby. The author wishes to emphasize that he has not had access to certain English and American papers published during the war.

(2) K. Freudenberg, *Chem.-Ztg.*, **60**, 853, 875 (1936).

(3) H. Berlin, *J. Am. Chem. Soc.*, **48**, 1107, 2627 (1926).

(4) See R. W. Kerr, editor, "Chemistry and Industry of Starch," Academic Press, Inc., New York, N. Y. (1944).

(5) C. O'Sullivan, *J. Chem. Soc.*, **29**, 478 (1876).

(1) It has been supposed that the amylase action leads to an equilibrium between starch and maltose or between maltose and certain dextrins. This is not the case, for if the limit dextrins are isolated and treated with amylase no action or at most only an extremely slow action is observed.

(2) The amylases only attack comparatively long chains and the degraded portions of the molecules remain because the chains are too short to be attacked. This explanation has been discussed in Kerr's text.[4]

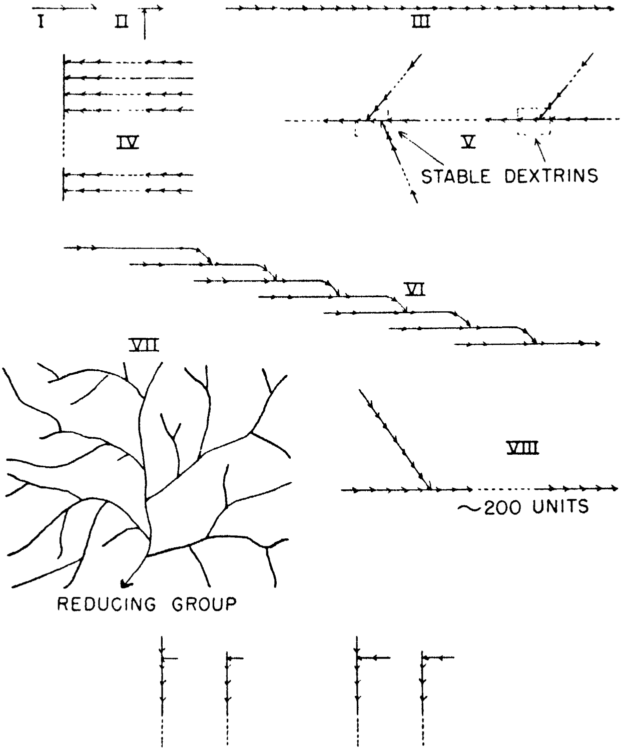

FIG. 1.—I, maltose; II, isomaltose; III, original Haworth formula; IV, Staudinger formula; V, origin of stable dextrins; VI, modified Haworth formula; VII, Meyer formula; VIII, branched molecule; IX–XII, end groups.

It can be shown, as will be discussed in detail later, that hexasaccharides with maltose linkages are completely converted to maltose by all amylases, and that if a hexasaccharide of this type is transformed into a mixture of saccharides with 1 to 6 units by acid hydrolysis, this mixture is completely saccharified. Thus when a chain of D-glucose units contains only maltose linkages, it is completely broken down by the amylases irrespective of the chain length, and the velocity is for most amylases much the same as in the case of starch.

In this connection it should be pointed out that *maltotriose*, a trisaccharide with two maltose linkages, is not attacked by β-amylase, but is *fermented* by common yeasts.[6] This has possibly caused some confusion in the chemistry of starch degradation.

The formation of trisaccharides during degradation of potato starch by pancreatic amylase has been studied by Blom and Rosted.[6a] The nature of these trisaccharides has not been established, but it seems probable that maltotriose is the main constituent. The sugar was fermented by *Saccharomyces cerevisiae* but not by *S. uvarum.*

(3) A very peculiar explanation for the formation not only of the limit dextrins but also of maltose has been given by Pringsheim.[7] He regarded D-glucose as the sole primary product of the amylase action and all other products as formed by spontaneous combination of two or more labile D-glucose residues. But it is certainly superfluous now to discuss this explanation when we know, for instance, that amylose and other saccharides with maltose linkages yield no limit dextrins. We may now take for granted that maltose and the limit dextrins are primary products formed by rupture of certain D-glucosidic linkages of the polysaccharide molecules.

It must be conceded that there is one case where the products of amylase action are probably not primary products formed in this way. This is the degradation of starch with the amylase from *Bacillus macerans.* The cycloamyloses formed by this enzyme are probably not preformed in the starch (see also Section II, 10).

Now as the limit dextrins are stable toward the amylases, it seems sound to assume that they are built according to another scheme than are those parts of the starch which yield maltose. In order to explain the incomplete saccharification and the formation of the limit dextrins, the author has assumed[8,9] that, although the starch molecules are built substantially according to the maltose scheme, there are at certain intervals *anomalies* of one kind or other which constitute hindrances to the enzyme action. Since in most cases 70 to 80% of maltose is formed from starch, it must be concluded that the normal action of the amylases is the opening of 1,4-α-D-glucosidic links. When the constitution of the chain differs in some way from that of maltose, the enzyme cannot attach itself to the "anomalous" part of the chain and no hydrolysis occurs. The author assumed that when, for instance, β-amylase acts on starch, the

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(6) K. Myrbäck and Elsa Leissner, *Arkiv Kemi, Mineral. Geol.*, **17A,** No. 18 (1943).

(6a) J. Blom and C. O. Rosted, *Acta Chem. Scand.*, **1,** 233 (1947).

(7) H. Pringsheim, "Die Polysaccharide," Julius Springer, Berlin (1931).

(8) K Myrbäck, *Current Sci.*, **6,** 47 (1937).

(9) K. Myrbäck and K. Ahlborg, *Svensk Kem. Tid.*, **49,** 216 (1937).

enzyme attaches itself to the end groups of the chains, setting free maltose molecules until it reaches a point in the chain where the constitution differs from the simple Haworth formula. At this point the enzyme action ceases, leaving the remainder of the molecule as a limit dextrin. Several types of anomalies were considered,[8,9] such as substitution by phosphoric acid, but especially D-glucosidic linkages in positions other than the usual 1,4-position. These probably represent chain ramifications (Fig. 1, formula V).

It may be stated immediately that if this theory of the origin of the limit dextrins is correct, then it must be concluded that the limit dextrins contain the "anomalies" in the starch molecules. This is the case since all limit dextrins, hitherto examined in detail, have been shown to contain at least one D-glucosidic linkage in the 1,6-position (isomaltose linkage).

It is quite clear that the original starch formula of Haworth is not in conformity with the most conspicuous qualities of starch. For instance, starch has practically no reducing properties. The ultracentrifugal assay gives very high values for the molecular weight, and the high viscosities of starch solutions also point to a high degree of polymerization. Esters and ethers of starch show the same high degree of polymerization as does starch itself, and from the esters starch may be regenerated with an unaltered degree of polymerization. From these results Staudinger[10] has concluded that the molecules of starch, glycogen and other polysaccharides are true macromolecules containing, in the case of starch, hundreds or thousands of D-glucose units. The end group assay, however, gives an average chain length of only about 25 units, although it must be kept in mind that the calculations of chain lengths from the end group values is possible only under the assumption that the chains are simple and unbranched. In order to obtain consistency between the end group values and the high degree of polymerization, Staudinger and Husemann[11] proposed a branched formulation of starch and glycogen, later sometimes termed the "comb" formulation. These authors assume the molecules to consist of a relatively short "principal chain" with numerous side chains attached to the principal chain by 1,6-, 1,3- and 1,2-α-D-glucosidic linkages (Fig. 1, formula IV). It is noteworthy that Staudinger and Husemann mention the possibility of multiple branching of the chains.

Haworth, Hirst and Isherwood,[12] in 1937, proposed a "laminated" formulation of the starch molecule (Fig. 1, formula VI), in which there

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(10) H. Staudinger, "Organische Kolloidchemie," Vol. 93 of "Die Wissenschaft," F. Vieweg & Sohn, Braunschweig (1940).

(11) H. Staudinger and Erika Husemann, *Ann.*, **527**, 195 (1937).

(12) W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 577 (1937).

is no "principal chain" but the reducing group of each individual chain is attached, on the average, to the central D-glucose member of the next chain. The average length of the chains should be, as in the Staudinger formula, about 25 D-glucose units.

Recently the old question of "amylose" and "amylopectin" seems to have received its final settlement. Meyer[13,14] and others[14a] have employed extraction of the swollen but intact granules with successive portions of water, a procedure that yields an amylose still contaminated with amylopectin. The method introduced by Schoch[14a,15,16] was a great improvement, and yielded pure amylose. It is now clear that most starches can be divided into two fractions of different structure: amylose and amylopectin.

Amylose gives in the end group assay only traces of tetramethyl-D-glucopyranose.[17,18] This and other facts, among them the fact that amylose is completely saccharified by β-amylase, show that amylose, or in any case corn starch amylose, has a linear molecule without branching. The degree of polymerization seems to be 200 to 300 D-glucose units. It is not quite clear whether the amylose fractions from other starches, as for instance potato starch, are quite unbranched, but the degree of ramification is certainly very low. Possibly there are in some starches all kinds of intermediates from amylose to amylopectin.

Amylopectin is a polysacccharide with a very high molecular weight. The yield of tetramethyl-D-glucopyranose from the methylated polysaccharide shows that the molecules must be highly branched.

Because of these new observations, we must conclude that the starch formulas discussed above apply to the amylopectin, whereas the corn amylose has the original Haworth formula but with a degree of polymerization of 200 to 300 instead of 25. The remaining question is the establishment of the pattern of ramification in amylopectin and in glycogen.

If a polysaccharide of the amylopectin type is branched, the methylated polysaccharide must on hydrolysis yield not only tri- and tetramethyl-D-glucose but also dimethyl-D-glucose. Freudenberg[19,20,21] states

(13) K. H. Meyer, W. Brentano and P. Bernfeld, *Helv. Chim. Acta*, **23**, 845 (1940).

(14) K. H. Meyer, P. Bernfeld and E. Wolff, *Helv. Chim. Acta*, **23**, 854 (1940).

(14*a*) See T. J. Schoch, *Advances in Carbohydrate Chem.*, **1**, 247 (1945).

(15) T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941).

(16) T. J. Schoch, *J. Am. Chem. Soc.*, **64**, 2957 (1942).

(17) K. H. Meyer, M. Wertheim and P. Bernfeld, *Helv. Chim. Acta*, **23**, 865 (1940).

(18) K. Hess and B. Krajnc, *Ber.*, **73**, 976 (1940).

(19) K. Freudenberg and H. Boppel, *Ber.*, **73**, 609 (1940).

(20) K. Freudenberg and H. Boppel, *Naturwissenschaften*, **28**, 264 (1940).

(21) K. Freudenberg, *Ber.*, **76A**, 71 (1943).

that methylated starch yields on hydrolysis 90% 2,3,6-trimethyl-D-glucose, 5% tetramethyl-D-glucopyranose and 5% or a little more dimethyl-D-glucose, mainly 2,3-dimethyl-D-glucose, and concludes that the point of branching is the hydroxyl group in the position 6. As no 2,3,4-trimethyl-D-glucose could be detected, he further concludes that 1,6-linkages without branching do not occur in starch.[21] Other investigators, however, have found less dimethyl-D-glucose. Caldwell and Hixon,[22] after hydrolysis of the methylated β-dextrin (produced by the action of β-amylase on starch), which should contain about 10% end groups, found only traces of dimethyl-D-glucose. On the other hand, Haworth[12] obtained dimethyl-D-glucose from methylated glycogen in approximately the same yield as tetramethyl-D-glucopyranose.

If we assume that the branching points of the amylopectin molecules are chiefly the anomalies which cause the formation of the limit dextrins, it might be expected that certain of the limit dextrins should likewise contain branchings. But this assumption is not necessarily valid because the amylase action may proceed until one of the branches is completely saccharified. In the limit dextrins hitherto investigated in detail, no points of branching have been found, but there is always a 1,6-D-glucosidic linkage. Therefore, our results concerning the constitution of the limit dextrins so far do not prove that the amylopectin molecule is branched, but only that 1,6-D-glucosidic linkages are present. The occurrence of these appears to be evident also from the fact that isomaltose (6-α-D-glucosyl-D-glucose) may be produced from starch by acid hydrolysis under such conditions that no reversion is possible.[23] But the experiments of Freudenberg[21] seem to exclude the possibility of 1,6-D-glucosidic linkages without branching. Moreover, it is very difficult to understand the action of the amylases if it be assumed that the amylopectin molecule has no side chains but has 1,6-linkages at intervals in the main chain. The only possibility seems to be the assumption that the amylopectin is highly branched. The formulas proposed by Staudinger (Fig. 1, formula IV) and by Haworth (formula VI) have in common a certain regularity. Quite irregular is formula VII proposed by Meyer,[24] which is based on experiments with β-amylase and an "α-D-glucosidase" from yeast. A formula of this type seems to give a satisfactory explanation of the action of the amylases. It can be pointed out at once that if the Staudinger formula is to explain the action of the β-amylase, it is neces-

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(22) C. G. Caldwell and R. M. Hixon, *J. Am. Chem. Soc.*, **63**, 2876 (1941).

(23) K. Ahlborg and K. Myrbäck, *Biochem. Z.*, **308**, 187 (1941).

(24) K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **23**, 875 (1940); K. H. Meyer (L. E. R. Picken, translator), "Natural and Synthetic High Polymers," Interscience Publishers, Inc., New York (1942).

sary to assume that the side chains are shorter (10 to 12 units) and are situated at greater intervals (8 to 10 units) on the principal chain. The formula of Haworth (VI) explains the action of the β-amylase if it be assumed that the individual chains are attached, on the average, to the central D-glucose unit of the next chain. On the other hand, in order to explain the action of the α-amylase, Haworth, Kitchen and Peat[25] have resorted to some provisional albeit rather improbable suppositions.

In the theories mentioned above concerning the pattern of the amylopectin molecule, our present knowledge of the enzymic synthesis of starch has not entered into the discussion. To the author it seems necessary now to consider the proposed formulas also from this point of view. In recent years, great progress has been achieved in the enzymic synthesis of starch and glycogen (Cori, Hanes and others[4]) and it is clear by now that the synthesis of these polysaccharides is the reverse of the phosphorolysis yielding α-D-glucopyranose 1-phosphate[25a] (Cori ester). For the synthesis the presence of a "germ" or activator is necessary. Starch, glycogen and all saccharides with maltose linkages having at least three D-glucose units[26] act as activators and the synthesis evidently takes place in such a way that the enzyme attaches a new D-glucose unit in the form of the Cori ester to the end group of the existing chain of the activator with the liberation of phosphoric acid. In the early experiments with the plant phosphorylases, the attachment of the new unit was in the 4-position since the polysaccharides synthesized seemed to consist exclusively of amylose.[4] Haworth, Peat and coworkers[26a] were able to produce an amylopectin type of molecule with an enzyme system found in the potato.

If ramified molecules exist in starch and in glycogen, it must be concluded that, under certain conditions, the attachment of the new D-glucose unit sometimes also takes place in the 6-position. In the case of amylopectin, the result is a polysaccharide with something like 5.5% 1,6-α-D-glucosidic linkages. Haworth, Peat and Bourne[26a] have shown that in addition to the phosphorylase discovered by Hanes (termed "P-enzyme") there is in the potato a second enzyme, termed "Q-enzyme." The combined action of the two enzymes leads to the formation of branched-chain

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(25) W. N. Haworth, H. Kitchen and S. Peat, *J. Chem. Soc.*, 619 (1943).

(25*a*) M. L. Wolfrom and D. E. Pletcher, *J. Am. Chem. Soc.*, **63**, 1050 (1941); M. L. Wolfrom, C. S. Smith, D. E. Pletcher and A. E. Brown, *ibid.*, **64**, 23 (1942).

(26) C. Weibull and A. Tiselius, *Arkiv Kemi, Mineral. Geol.*, **19A**, No. 19 (1945); G. T. Cori, M. A. Swanson and C. F. Cori, *Federation Proc.*, **4**, 234 (1945); P. H. Hidy and H. G. Day, *J. Biol. Chem.*, **152**, 477 (1944); **160**, 273 (1945); J. B. Sumner, G. F. Somers and Eleanor Sisler, *J. Biol. Chem.*, **152**, 479 (1944).

(26*a*) W. N. Haworth, S. Peat and E. J. Bourne, *Nature*, **154**, 236 (1944); E. J. Bourne and S. Peat, *J. Chem. Soc.*, 877 (1945); E. J. Bourne, A. Macey and S. Peat, *ibid.*, 882 (1945).

molecules. The authors postulate the formation of a "pseudo-amylose," a simple chain polysaccharide with about 20 D-glucose units, which they regard as the substrate of the Q-enzyme. A synthesis proceeding in this way would yield a polysaccharide, the individual chains of which would have an average length of 20 D-glucose units as in the formulas of Staudinger and Haworth, Kitchen and Peat. But it is not clear why every chain should have only one side chain, that is, why the ramification should be regular.

An attempt has been made by Myrbäck and Sillén[27,28,29,30] to calculate, employing only the simplest working hypotheses, the constitution of a polysaccharide, the molecules of which grow from germinal points as indicated above. It seems a fair assumption that, for instance, in a certain plant under certain external conditions, the probability of attachment of a new D-glucose unit in the position 4 of the end group of the existing chain, which means lengthening of the chain, should be in constant ratio with the probability of attachment in the position 6, which means ramification of the chain. Assuming that all end groups are equal in this respect and that the probability of attachment in the 6-position is the same for all free 6-positions, it is possible to describe[27] the ramified molecule formed. The molecule will have a quite irregular pattern of ramification, and all values of chain lengths will be averages.

If the probability of attachment in the 4-position, that is the probability of lengthening of the chain in the time dt is kdt, then the probability of branching of the chain is γkdt, if γ is the constant ratio as defined above. If N is the total number of D-glucose units in the molecule formed, and B is the number of branching points, it can be shown that the *degree of ramification*, $\beta = B/N$, rapidly approaches a definite limit value

$$\beta = \frac{\sqrt{\gamma}}{1 + \sqrt{\gamma}}$$

when t increases. This means that after a comparatively short time of growing, the molecule has acquired a degree of ramification which does not change significantly on further increase of the degree of polymerization. This is in agreement with the fact that, according to Meyer, amylopectins of varying degrees of polymerization have the same degree of ramification or end group content.

In a branched molecule all chains with free ends except one have nonreducing end groups. These chains we call *end chains*. The chains

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(27) L. G. Sillén and K. Myrbäck, *Svensk Kem. Tid.*, **55**, 294 (1943).

(28) K. Myrbäck and L. G. Sillén, *Svensk Kem. Tid.*, **55**, 311 (1943).

(29) L. G. Sillén and K. Myrbäck, *Svensk Kem. Tid.*, **55**, 354 (1943).

(30) K. Myrbäck and L. G. Sillén, *Svensk Kem. Tid.*, **56**, 60 (1944).

without free ends, existing between ramifications (or other anomalies), are called *interior chains.* If the number of D-glucose units in end chains is $Z_1 = \zeta_1 N$, then

$$\zeta_1 = 0.6557 - 0.9371\beta + 0.0651\beta^2 - 0.0654\beta^3 \cdots \quad (\beta > 0),$$

for $\beta = 0$, $\zeta_1 = 1$. The average length of the end chains is

$$\bar{n}_e = \frac{\zeta_1}{\beta}$$

and the average length of the interior chains is

$$\bar{n}_i = \frac{1}{\beta}(1 - 2\beta - \zeta_1).$$

If we assume that β for amylopectin is $1/18$ and for glycogen $1/11$, we find the following.

	γ	ζ_1	$\bar{n}_e$	$\bar{n}_i$
For amylopectin	0.00346	0.6045	10.9	5.1
For glycogen	0.01000	0.5711	6.3	2.8

It may be stated that the values calculated for the mean chain lengths agree well with the values predicted by Meyer[31] on the basis of experiments with amylases.

It is also possible to calculate the distribution of the D-glucose units on chains of different lengths.[29] The values calculated for amylopectin and glycogen are illustrated in Fig. 2. It is seen from this figure that if the calculation is substantially correct there should be among the end chains of the amylopectin an appreciable portion having chain lengths of more than 30 units whereas in the glycogen, end chains of this length should be rare.

In the following discussion, formulas for amylopectin and glycogen based on these calculations will be used as working hypotheses for the understanding of the enzymic degradation of the polysaccharides.

It has been pointed out by Kerr[4] that a molecule having the formula of Meyer should have a more or less spherical shape. This seems to be the case for glycogen, whereas amylopectin solutions have a very high viscosity suggesting a highly elongated form. To the author it seems possible that the spherical shape of the glycogen molecule results from the growing of the molecule as suggested above, the growing taking place in

(31) K. H. Meyer, *Naturwissenschaften,* **29,** 287 (1941).

solution where the molecule can assume the natural shape. In the case of the amylopectin, however, it seems possible that the growth of the molecules takes place on the surface of the granules, giving a faster growth in the radial direction. The growth would, as it were, take place as in a small tube resulting in an oblong shape of the molecule.[31a] It might even be possible that circumstances like these cause the formation of quite unbranched molecules.

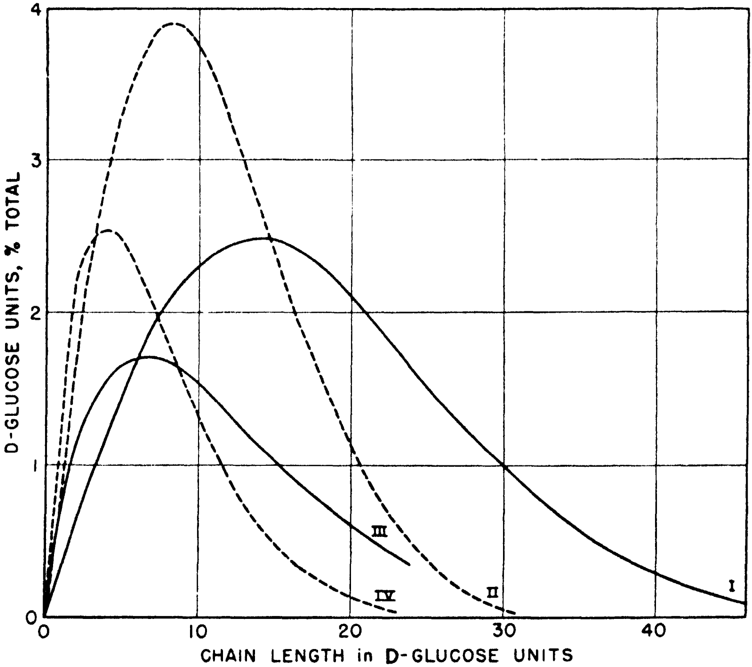

FIG. 2.—Distribution of D-glucose units on chains of different lengths. I, end chains of amylopectin; II, interior chains of amylopectin; III, end chains of glycogen; IV, interior chains of glycogen.

II. The Enzymic Degradation of Starch and Glycogen

1. *Action of β-Amylase*

After earlier workers had indicated the existence of several amylases in malt, conclusive evidence that the "malt amylase" is composed of at least two starch-splitting enzymes was furnished by Ohlsson.[32,33] Both enzymes are capable of attacking starch and glycogen, but their action is very different. One enzyme, termed saccharogenic amylase by Ohls-

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(31a) K. Myrbäck, *Svensk Papperstidn.*, **50**, No. 11b (1947).

(32) E. Ohlsson, *Compt. rend. soc. biol.*, **87**, 1183 (1922).

(33) E. Ohlsson, *Compt. rend. trav. lab. Carlsberg*, **16**, No. 7 (1926).

son, gives 60% maltose from starch and a residue which is stained blue or violet by iodine. The other enzyme, termed dextrinogenic amylase, rapidly converts starch to products which give no color with iodine and at the same time only small amounts of fermentable sugars are formed. It is concluded that the enzyme breaks up the starch molecules into dextrins of too low a degree of polymerization to give any color with iodine. Ohlsson was later able to show[34] that the saccharogenic amylase is a *β-amylase* and the dextrinogenic amylase an *α-amylase*, in the sense introduced by Kuhn.[35] The β-amylase occurring in higher plants is the only known β-amylase.

The best source of the β-amylase is ungerminated barley (or wheat, oats, rye and soybeans) from which very active solutions may be prepared by simple extraction with cold water and a little toluene. A much higher yield of the enzyme can be obtained by extraction with papain solution.[36,37,38,39] In the crude extracts, the β-amylase very often is contaminated with traces of α-amylase. This seems not to have been properly taken into consideration by several investigators. α-Amylase is present when the enzyme preparations acting on starch produce products which are not colored by iodine. Further, in the presence of α-amylase more than 61% of maltose is formed from potato starch. The yield of maltose should be determined (after inactivation of the enzyme) not only by reduction methods but also by fermentation or other experiments that distinguish maltose and D-glucose. The enzyme solution may contain maltase and this will result in a high apparent sugar value. β-Amylase free from α-amylase gives with potato starch a sharp limit of saccharification at 61% maltose (calculated on the theoretical amount, 105.5%, of the starch). Blom[40] and coworkers have given a reliable method for preparing pure β-amylase; the α-amylase is destroyed by the addition of acetic acid and storage of the solution at 0° and *p*H 3.6.

The saccharification of potato starch with pure β-amylase yields 61% of maltose and not more. In a series of experiments we obtained as the mean value from the reduction, 61.1%, and from the fermentation experiments, 60.8%. The reduction was (as in most experiments in this laboratory) determined by a modification[41,42] of the iodometric method of

(34) E. Ohlsson, *Z. physiol. Chem.*, **189**, 17 (1930).
(35) R. Kuhn, *Ann.*, **443**, 1 (1925).
(36) K. Myrbäck and Signe Myrbäck, *Biochem. Z.*, **285**, 282 (1936).
(37) K. Myrbäck and B. Örtenblad, *Biochem. Z.*, **293**, 107 (1937).
(38) K. Myrbäck and B. Örtenblad, *Enzymologia*, **7**, 176 (1939).
(39) K. Myrbäck and B. Örtenblad, *Enzymologia*, **9**, 43 (1940).
(40) J. Blom, A. Bak and B. Braae, *Z. physiol. Chem.*, **241**, 273 (1936).
(41) K. Myrbäck and B. Örtenblad, *Svensk Kem. Tid.*, **50**, 72 (1938).
(42) K. Myrbäck and Ebba Gyllensvärd, *Svensk Kem. Tid.*, **53**, 461 (1941).

Willstätter and Schudel.[43] From the reduction values the amount of maltose was calculated. D-Glucose was absent. The presence of very small amounts of D-glucose in the presence of large amounts of maltose can be shown by fermentation experiments with *Saccharomyces fragilis* or other "milk sugar yeasts" which ferment D-glucose but not maltose. Ordinary Swedish bakers' yeast ferments D-glucose much more rapidly than maltose, so that a determination of both sugars is possible.[44,45] Experiments with different starches show that β-amylase does not in any case yield D-glucose.[45]

Blom and coworkers[40] have found the limit of saccharification to be 53% at *p*H 3.4. Higher values are attributed to traces of α-amylase. This question has been exhaustively discussed by Hopkins.[45a] It should be pointed out that a different degree of saccharification at different *p*H's seems understandable if one assumes that the influence of the proximity of branching points or other anomalies on the β-amylase varies with the *p*H. Hopkins and Cooper[45b] find the limit value 56%. They point out that barley amylase very often is contaminated with amylases from bacteria or moulds present on the grains.

The β-amylase liberates the maltose in the β-configuration.[35,46,47] This is no proof for the occurrence of β-D-glucosidic linkages in starch. On the other hand, it is not known why the β-amylase, in contrast to the α-amylases, causes an inversion of the configuration.

In the fermentation industries it has been known for a long time that a better yield of fermentable sugar or alcohol, respectively, is obtained if the "malt amylase" is allowed to act during the fermentation. This has sometimes been interpreted as an activation of the amylases by the yeast. Hopkins, Cope and Green[48] find that β-amylase acting on starch or glycogen is activated in this way by living yeast, the yield of alcohol suggesting that the saccharification in the presence of yeast proceeds to 80–90%. The action of the yeast is not easy to understand. As very great amounts of the enzyme from barley gave a conversion without yeast of about 97%, it must be concluded that traces of α-amylase were present.

If potato starch contains about 20% amylose and if the amylose is saccharified to 100%, it is clear that amylopectin is saccharified by β-amylase to about 50%. This is confirmed in experiments with amyl-

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(43) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918).

(44) K. Myrbäck, *Biochem. Z.*, **304**, 147 (1940).

(45) K. Myrbäck, *Biochem. Z.*, **307**, 140 (1941).

(45*a*) R. H. Hopkins, *Advances in Enzymol.*, **6**, 389 (1946).

(45*b*) R. H. Hopkins and T. F. S. Cooper, *J. Inst. Brewing*, **52**, 188 (1946).

(46) H. v. Euler and Karin Helleberg, *Z. physiol. Chem.*, **139**, 24 (1924).

(47) R. Kuhn, *Ber.*, **57**, 1965 (1924).

(48) R. H. Hopkins, G. F. Cope and J. W. Green, *J. Inst. Brewing*, **39**, 487 (1933).

opectin freed from amylose by precipitation with butanol.[15,16] Thus it must be concluded that the β-amylase has the capacity of attacking the non-reducing end groups of the end chains. That the reducing end does not play any significant role for the action on starch is shown in experiments with starch preparations previously treated with hypoiodite,[49,50] by which presumably all existent reducing groups have been turned into carboxyl groups. Such preparations are saccharified with the same velocity and to the same extent as normal starch.

"Normal" chains, that is, chains with 1,4-α-D-glucosidic linkages exclusively (Haworth formula), are saccharified by the β-amylase to 100% irrespective of the chain length. Even very short chains are attacked, for instance, chains with 6 or 4 D-glucose units. Normal chains with 5 to 7 units can be isolated in good yield from the products of the action of the malt α-amylase, if the reaction is stopped at the right moment (see below). These saccharides are completely split into fermentable sugar by the β-amylase.[51] If saccharides of this type are partially hydrolyzed by acid into mixtures of saccharides with 1 to 7 units, such mixtures are also converted by the β-amylase to 100% fermentable sugar. The main product is also in this case always maltose; D-glucose is not formed. It must be concluded that maltotriose is formed from chains with an uneven number of D-glucose units. As pointed out above, this sugar is not attacked by the β-amylase but is fermented by yeast.

In this connection it may be mentioned that many ordinary yeasts ferment, generally very slowly, isomaltose and trisaccharides (limit dextrins) with one maltose and one isomaltose linkage.[44,52] Dried yeasts ferment starch and limit dextrins with a fairly high velocity.[53] This fermentation is probably not due to a hydrolysis of the saccharides to D-glucose or maltose, but to a phosphorolysis to the Cori ester (α-D-glucopyranose 1-phosphate).

In the literature there has been a certain disagreement as to whether amylose is saccharified completely by β-amylase. The experiments are complicated by the fact that amylose solutions have a tendency to deposit amylose (retrograde) in which state it is not attacked by the enzyme.[53a] But if a sufficient amount of enzyme is used the amylose is, without

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(49) K. Myrbäck, *Biochem. Z.*, **285**, 290 (1936).

(50) B. Örtenblad and K. Myrbäck, *Biochem. Z.*, **307**, 129 (1941).

(51) K. Myrbäck and K. Ahlborg, *Biochem. Z.*, **311**, 213 (1942).

(52) K. Myrbäck, *Svensk Kem. Tid.*, **53**, 67 (1941).

(53) K. Myrbäck, B. Örtenblad and K. Ahlborg, *Enzymologia*, **3**, 210 (1937).

(53*a*) O. E. Stamberg and C. H. Bailey, *Cereal Chem.*, **16**, 330 (1939); R. H. Hopkins, E. G. Stopher and D. E. Dolby, *J. Inst. Brewing* **46**, 426 (1940).

question, completely saccharified.[54,55] The complete saccharification by β-amylase is demonstrated in such experiments only if pure β-amylase free from α-amylase and from maltase is used. In the author's opinion, the fact that several investigators report an incomplete saccharification of amylose by β-amylase is connected with the retrogradation of the amylose solutions. Or, in some cases, it may be that some of the amylose molecules have a few side chains. If a molecule has one side chain situated as in Fig. 1, formula VIII, it will probably be saccharified only to about 5% by β-amylase. It is true that this molecule has twice as high an end group content as an unbranched molecule, but if the number of branched molecules is not high it will be impossible to detect their presence from the end group values only. In any case, starch degradation products with no anomalies and with no tendency to retrogradation are completely saccharified. It is perhaps unnecessary to mention that a complete saccharification is not obtained with most preparations formerly called amylose. For instance, amylose prepared according to Ling in experiments by the author gave only the same yield of maltose as starch itself.[56] The same result is reported by Hirst, Plant and Wilkinson.[57]

Short normal chains containing 1,4-α-D-glucosidic linkages only are completely saccharified even if the aldehyde group is oxidized to a carboxyl group. Such experiments were conducted[58] with a maltohexaose and the corresponding hexaonic acid. The hexaose gave with β-amylase 100% maltose, the hexaonic acid about 60%, showing that the acid was split into two molecules of maltose and one molecule of maltobionic acid. The first mole of maltose was liberated at a higher velocity than the second. The enzyme action is quite independent of the reducing group; it attacks the chains from the non-reducing end group. An α-dextrin (see below) containing one or more "anomalies" (1,6-α-D-glucosidic linkages) was treated in the same manner. Dextrin and dextrinic acid were saccharified to the same extent. This was determined by the position of the anomaly, that is, the length of the chain between the non-reducing end group and the "anomaly."

When starch, amylopectin or glycogen is saccharified by β-amylase and the maltose is removed, there remains the limit dextrin, called β-amylase dextrin, "Grenzdextrin," α-amylodextrin or in publications from the author's laboratory, simply *β-dextrin*. It gives viscous solutions and is colored blue or violet by iodine. After precipitation with alcohol

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(54) K. Myrbäck and Walborg Thorsell, *Svensk Kem. Tid.*, **54**, 50 (1942).

(55) G. G. Freeman and R. H. Hopkins, *Biochem. J.*, **30**, 446 (1936); K. H. Meyer, P. Bernfeld and J. Press, *Helv. Chim. Acta*, **23**, 1465 (1940); **24**, 50 (1941).

(56) K. Myrbäck and Signe Myrbäck, *Svensk Kem. Tid.*, **45**, 230 (1933).

(57) E. L. Hirst, M. M. T. Plant and M. D. Wilkinson, *J. Chem. Soc.*, 2375 (1932).

(58) K. Myrbäck and Gunhild Nycander, *Biochem. Z.*, **311**, 234 (1942).

and drying it is in many cases only partly soluble in boiling water. No products of low molecular weight other than maltose are formed.[59] The degree of polymerization of the β-dextrin is very high. The author has in some cases determined the diffusion constant of the soluble part. The values are very low.[9,60] The β-dextrin from wheat starch had $k_{20^\circ} = 0.0106$. If the molecular weight is calculated from the Euler formula (which definitely is not allowable) we get $M = 440{,}000$. The value only shows that the degree of polymerization is very high and presumably of the same order of magnitude as that of wheat starch. Of greater interest are determinations of the molecular weight of β-dextrins derived from degraded starches with low molecular weights. A Zulkowski starch with $M = 25{,}000$ gave a β-dextrin with $M = 19{,}000$ and a soluble Lintner starch with $M = 12{,}000$ gave a β-dextrin with $M = 8{,}000$. It is clear that the β-amylase does not break up the starch molecules; it only shortens the end chains, leaving the rest of the molecules as a limit dextrin of a high degree of polymerization. Naturally the β-dextrin is heterogeneous in respect to molecular weight.

The β-dextrin (α-amylodextrin) has an end group content of 10–11%.[61] Now if starch yields 40% of the limit dextrin, its end group content should be 11.3% if the starch has 4.5% end groups. Thus we can conclude that no, or only few, end groups vanish under the influence of β-amylase. Meyer[24,62] has concluded that the β-dextrin remaining after the action of the β-amylase must contain end groups of the types shown in Fig. 1, formulas IX and X. As far as the author can determine there is an equal probability that groups XI and XII are formed. IX and XI must yield two moles of tetramethyl-D-glucose, but X and XII only one. Consequently, if all end chains in the amylopectin are reduced to groups like IX–XII, the β-dextrin should be expected to have only about half the end group content calculated as above. The values found by Haworth, Hirst, Kitchen and Peat[61] therefore support the view, expressed by Meyer, that the amylopectin can not have simple branchings as in the Staudinger or Haworth formulas (IV and VI in Fig. 1). If the molecules have multiple branchings as in the formulas proposed by Meyer and by Myrbäck and Sillén, only a relatively small number of the branching points will vanish under the action of β-amylase.

All of the phosphoric acid associated with potato starch is found in the β-dextrin.[9,60,63]

(59) G. G. Freeman and R. H. Hopkins, *Biochem. J.*, **30**, 442 (1936).

(60) K. Myrbäck, *Biochem. Z.*, **297**, 160 (1938).

(61) W. N. Haworth, E. L. Hirst, H. Kitchen and S. Peat, *J. Chem. Soc.*, 791 (1937).

(62) K. H. Meyer, *Naturwissenschaften*, **28**, 564 (1940).

(63) K. Myrbäck and B. Kihlberg, *Biochem. Z.*, **315**, 250 (1943).

As mentioned above, potato starch is saccharified to 61% with β-amylase. Only a few experiments on other starches seem to have been made with pure β-amylase. Soluble starches from potato starch are saccharified to the same extent.[63a,63b] For strongly degraded starch preparations the limit of saccharification is lower. Zulkowski starch from potato starch gives 60% maltose, but lower degradation products of the same type give a lower yield (Table I). (On the nature of the action of glycerol, see Berner and Melhus.[64]) This is in accordance with the fact that the 1,6-D-glucosidic linkages in starch are hydrolyzed with a lower velocity than the normal 1,4-linkages, leaving products with a higher "concentration" of anomalies.

TABLE I

Action of β-Amylase on Starch Preparations of Different Degrees of Polymerization

Starch preparation	*Mol. wt.*	*Per cent maltose*
Potato starch	>100,000	61
Lintner starch from potato starch	>100,000	60
Soluble "starch" from potato starch	12,000	59
Soluble "starch" (Staudinger)	5,800	61
Soluble "starch" (Staudinger)	2,300	49
Zulkowski starch from potato starch	25,000	60
"Hexahexosan Pringsheim"	3,200	40
"Hexahexosan Pringsheim"	1,700	40
"Trihexosan Pringsheim"	900	<10
"Trihexosan Pringsheim"	650	< 5
Product from degradation with glycerol	5,100	37
Product from degradation with glycerol	2,500	45
Product from degradation with glycerol	1,700	40
Product from degradation with glycerol	950	36
Product from degradation with glycerol	850	37

With some natural starches or with glycogen it is possible that the action of the β-amylase is stopped before all end chains are removed because the substrate molecule has so complicated a network of chains that the enzyme, which presumably has a large molecule, can not penetrate into the inner parts of the substrate molecule. This point has been stressed in the case of glycogen by Meyer and Jeanloz.[65] These authors degraded the substrate by a short treatment with hydrochloric acid to the extent that all end chains were accessible for the enzyme and a true saccharification limit was reached. Experiments with different starches and

(63a) B. Örtenblad and K. Myrbäck, *Svensk Kem. Tid.*, **50,** 168 (1938).

(63b) K. Myrbäck and B. Örtenblad, *Svensk Kem. Tid.*, **50,** 284 (1938).

(64) E. Berner and F. Melhus, *Ber.*, **66,** 1333 (1933).

(65) K. H. Meyer and R. Jeanloz, *Helv. Chim. Acta*, **26,** 1784 (1943).

with the corresponding amylopectins and pure β-amylase should be repeated inasmuch as the pattern of ramification of the molecules is to some extent clarified in this way.

If the molecules of amylopectin and glycogen are built up as suggested by Myrbäck and Sillén, it should be possible to calculate, from the degree of ramification (the end group value) the amount of maltose liberable by the β-amylase. As shown above the number of D-glucose units in the end-chains, ζ_1, can be calculated. But the amount of maltose liberated by the β-amylase must be smaller than this because the action of the enzyme in many cases must cease at a certain distance from the branching points. This is the case when the number of D-glucose units in the end chains is uneven. If we assume that all units are liberated as maltose in end chains containing an even number of units, and that in chains with an uneven number of units, one D-glucose entity remains in the limit dextrin, then the amount of maltose will be given by the following expression.[27]

$$\zeta_2 = 0.6557 - 1.4371\beta + 0.3151\beta^2 + 0.3154\beta^3 \cdots \quad (\beta > 0)$$

If we assume instead that in chains containing an uneven number of units, one D-glucose unit remains in the dextrin, and that in chains of even number, two units remain in the dextrin, then the amount of maltose will be as follows.

$$\zeta_3 = 0.6557 - 2.4371\beta + 1.8151\beta^2 + 1.8154\beta^3 \cdots \quad (\beta > 0)$$

The value of β for glycogen is approximately $1/11$. Then, $\zeta_2 = 0.53$ and $\zeta_3 = 0.46$. The first value agrees with that found by Meyer and Jeanloz[65] and should be the true saccharification limit of glycogen. Other authors have found lower values. Hopkins, Cope and Green[48] found 0.42 to 0.44.

Assuming that potato starch contains 23% amylose, the amylopectin must yield 49% maltose by action of the β-amylase. If the potato starch contains 5% end groups, the amylopectin should contain 6.5%. Taking this value, $\beta = 0.065$, we find $\zeta_2 = 0.56$ and $\zeta_3 = 0.51$. On the other hand, if we assume that potato starch contains branched molecules with an average degree of ramification expressed by $\beta = 0.05$, then $\zeta_2 = 0.59$ and $\zeta_3 = 0.54$.

a. Summary. It may be stated that the action of β-amylase on starch, amylopectin and glycogen is well understood if we assume that the amylose has long unbranched chains with 1,4-α-D-glucosidic linkages and that amylopectin and glycogen have repeatedly branched molecules. If we assume that the ramification is quite irregular and that the growth of the molecules takes place in a way tolerably like that which has been outlined above, it is possible to calculate approximately the amount of maltose that is liberated by the β-amylase. The calculated values have the right order of magnitude.

Now it is clear that the results concerning the action of β-amylase tend to show that amylopectin and glycogen have branched molecules but they do not show that the ramification is quite as irregular as assumed above. As pointed out previously, several other formulations may explain the behavior of the enzyme, if certain chain lengths be assumed. Staudinger and also Haworth have emphasized that they do not assume that all end chains have the same length or that they are situated at regular intervals on the other chains. But if we agree that the end chains and the interior chains have varying lengths, it seems to the author that side chains should appear not only on a principal chain but also on side chains, in which case we arrive at an irregular molecule with multiple branching.

The action of the β-amylase follows the monomolecular formula for the major part of its course. This means that the probability of liberation of a certain maltose unit is about the same, irrespective of its distance from a branching point or other anomaly. Since a reducing end group of a chain must be regarded as an anomaly, it is suggestive that hexaoses and tetraoses with maltose linkages are split with about the same velocity as starch itself. In a dextrinic acid, the carboxyl group certainly is an anomaly, but such acids with six or four D-glucose units are, as mentioned above, saccharified as expected. The rate of hydrolysis of the acid with four D-glucose units is somewhat lower, intimating that the anomaly has a certain effect but that it does not stop the enzyme from acting at a point two D-glucose units removed from the anomaly.

2. *Action of Malt α-Amylase*

This enzyme is prepared most simply by heating malt extracts to a temperature at which the β-amylase is inactivated. The temperature is not the same for all malt extracts. Impurities in the extracts seem to have a more or less protective action on the enzymes. In most cases, heating at 70° for about fifteen minutes will be suitable. Other methods of separating the enzymes are reviewed by Bamann and Myrbäck.[66]

It is not easy to show that a preparation of malt α-amylase is enzymically homogeneous and free from β-amylase and maltase. As will be shown later, the enzyme liberates D-glucose as a primary product from starch. Thus the appearance of D-glucose is not evidence of the presence of maltase. But the absence of this enzyme is most easily demonstrated

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(66) E. Bamann and K. Myrbäck, editors, "Die Methoden der Fermentforschung," G. Thieme Verlag, Leipzig, p. 1892 (1941); Academic Press, New York (1945).

in an experiment with maltose. It is much more difficult to show the absence of β-amylase. This can be done only by studying the curve representing the degree of hydrolysis plotted against time. As will be shown, this curve has a very peculiar shape. By comparing curves for enzyme preparations obtained by heating malt extracts for different periods of time and at different temperatures, it is possible to determine when the β-amylase is completely destroyed.

In contrast to the β-amylase, the malt α-amylase (and other liquefying amylases) very rapidly lower the viscosity of starch pastes. When the relative viscosity has dropped to half the original value, only about 0.1% of the D-glucosidic linkages in the starch have been hydrolyzed.[67] The malt α-amylase is a typical "liquefying" enzyme.

In this connection it is necessary to discuss the so-called amylophosphatase. According to Waldschmidt-Leitz and Mayer,[68] there is in barley and malt an enzyme which liberates phosphoric acid from starch with a simultaneous liquefaction but without production of reducing groups or alteration of the color reaction with iodine. Without actually denying the existence of an enzyme specifically liberating phosphoric acid from starch, the author wishes to emphasize that such an enzyme plays no significant role in the liquefaction of starch brought about by the ordinary amylase preparations. In fact the α-amylases liquefy starch without liberation of any phosphoric acid and the liquefaction is accompanied by a corresponding liberation of reducing groups. The liquefaction is, in other words, a true hydrolysis of D-glucosidic linkages in the polysaccharides.

The α-amylases rapidly alter the starch in such a way that the reaction mixture is no longer colored by iodine. With malt α-amylase and potato starch this is the case when about 7% of the D-glucosidic linkages are hydrolyzed, that is, when the apparent yield of maltose is 15–20%.[69] (The experiments were carried out with a preparation of soluble starch.)

When starch is treated with malt α-amylase and the production of reducing groups is estimated by oxidation with hypoiodite and the degree of hydrolysis (number of D-glucosidic linkages hydrolyzed expressed as % total initially present) is calculated, we obtain curve II in Fig. 3. This curve shows that the action of the enzyme divides itself into two phases. First, there is a rapid liberation of reducing groups accompanied by a complete loss of the high viscosity. The reaction mixture is not colored by iodine. Only small amounts of fermentable sugar are formed. This

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(67) K. Myrbäck and L. G. Gjörling, *Arkiv Kemi, Mineral. Geol.*, **20A**, No. 5 (1945).

(68) E. Waldschmidt-Leitz and K. Mayer, *Z. physiol. Chem.*, **236**, 168 (1935).

(69) K. Myrbäck and B. Lundberg, *Svensk Kem. Tid.*, **55**, 36 (1943).

phase of the reaction is termed the *dextrinization.* When about 17% of the D-glucosidic linkages of the starch are ruptured, the second phase begins. Herein the velocity, as measured by the increase in reducing groups, falls to a much lower value, amounting to some 6% of the velocity of the first phase. In this second phase, designated the *saccharification,* fermentable sugars are produced in increasing yield.

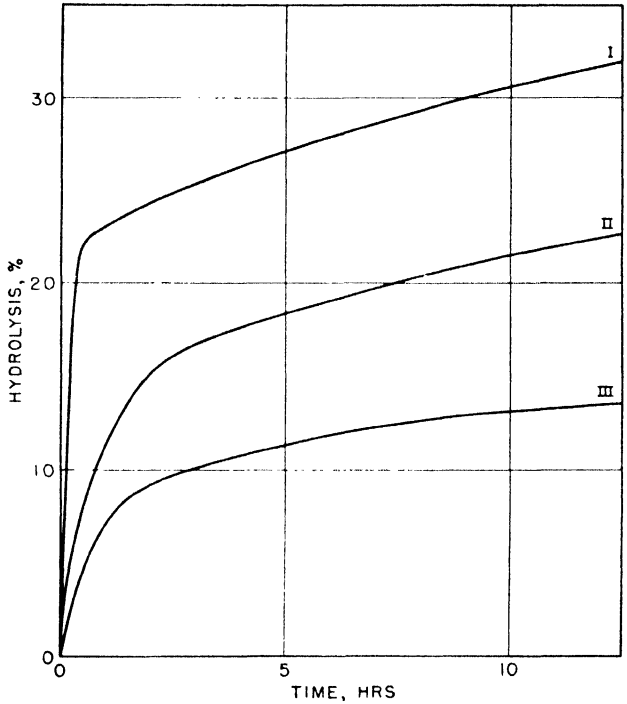

FIG. 3.—Hydrolysis by malt α-amylase. I, amylose; II, corn starch; III, β-dextrin (glycogen).

When examining curves of this type, one involuntarily inquires if both phases of the reaction are caused by the same enzyme or if the second phase is not an action of traces of β-amylase. But if malt extracts are heated to temperatures causing inactivation of the β-amylase, a variation of time and temperature does not alter the relation between the velocities of the two phases nor the relation between these velocities and the time necessary to change the starch so that it is not colored by iodine.[69] These relations also are not altered if the α-amylase is partly removed from the solutions by adsorption on bentonite, activated carbon, aluminum hydroxide, kaolin or silica.[70] Thus we must conclude that both saccharification and dextrinization are effected by the malt α-amylase. This must mean that the enzyme has the capacity of splitting some 17% of the D-glucosidic linkages in starch with a great velocity, and then proceeds to hydrolyze additional linkages with a much lower velocity. In order to understand this peculiar action of the enzyme, it is necessary to examine the nature of the products formed at different stages of the hydrolysis.

The following experiment was conducted with a soluble starch in order to obtain solutions clear enough to permit determination of the optical activity. Reduction was determined with hypoiodite and calculated as degree of hydrolysis. Maltose and D-glucose were determined by fermentation with bakers' yeast,[45] Table II. The enzyme was quite free from maltase, and we must conclude that D-glucose is a primary product of the action of the malt α-amylase. This was shown to be the case also with other starches. The production of D-glucose with "heat restricted" malt amylase was observed long ago by several workers in this field, as for example by Ling.[71,72,73] Most investigators seem to have attached no importance to this observation. Sherman and Punnet[74] found that amylase preparations from malt, pancreas and *Aspergillus oryzae* all give D-glucose in approximately the same yield. This must be due, at least partly, to an action of maltase. The presence of D-glucose in our experiments was demonstrated not only in fermentation experi-

TABLE II

Action of Malt α-Amylase on Soluble Starch

Time, min.	*Polarimetric reading*		*Hydrolysis, %*	*Fermentable sugar, %*	
	Before mutarotation	*After mutarotation*		D-*Glucose, %*	*Maltose + maltotriose, %*
2	+10.65°	+10.24°	17.0	2	10
12	10.30	9.80	21.5	3	13
22	10.19	9.76	24.3	5	14
60	9.62	9.22	29.0	8	19
180	8.70	8.62	36.0	11	41
390	8.50	8.42	40.8	12	52
24 hr.	8.08	8.08	45.7	13	64

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(70) K. Myrbäck and P. J. Palmcrantz, *Arkiv Kemi, Mineral. Geol.*, **18A**, No. 6 (1944).

(71) A. R. Ling and B. F. Davis, *J. Inst. Brewing*, **8**, 475 (1902).

(72) A. R. Ling, *J. Inst. Brewing*, **9**, 446 (1903).

(73) B. F. Davis and A. R. Ling, *J. Chem. Soc.*, **85**, 16 (1904).

(74) H. C. Sherman and P. W. Punnet, *J. Am. Chem. Soc.*, **38**, 1877 (1916).

ments with bakers' yeast but also in experiments with *Torula lactosa* Kluyver, which does not ferment maltose, and by the isolation of D-glucose phenylosazone. The liberation of D-glucose starts at once but ceases before the formation of maltose ceases. This means that D-glucose is set free from products of a relatively high degree of polymerization and not from the limit dextrins. Quite another thing is the liberation of D-glucose from limit dextrins after very long times of action of the amylase preparations (see below).

As mentioned above, β-amylase does not produce D-glucose. If starch is treated with a mixture of α- and β-amylase, the yield of D-glucose is much lower than with α-amylase alone. This means that the α-amylase produces D-glucose also from parts of the starch molecules that can be saccharified by the β-amylase.

It may be mentioned that the formation of D-glucose as a primary product is not characteristic of all α-amylases. Thus salivary amylase produces no D-glucose when free from maltase.[45] The production of D-glucose and the peculiar shape of the curve of hydrolysis, therefore, seems to be characteristic of the malt α-amylase.

Recent experiments from this laboratory show that pancreatic amylase when free from maltase causes no formation of D-glucose.

The course of the optical rotation during the action of the malt α-amylase shows that the enzyme, as pointed out by Ohlsson, liberates the hydrolysis products in the α-configuration. A simple calculation shows that this is the case not only with the fermentable sugar but with the dextrins as well.

When the degree of hydrolysis is 17%, corresponding to an apparent yield of maltose of about 35%, fermentation experiments show that only about 10% of fermentable sugar is produced. In the dextrinization, therefore, the enzyme breaks up the starch molecules into unfermentable parts having a fairly high reducing value (low molecular weight). These products of the dextrinization by malt α-amylase we call *α-dextrins.* The average molecular weight of the products of the dextrinization, including the fermentable sugars, is about 1100 (number degree of polymerization = 7). From this it is clear that malt α-amylase, in contrast to the β-amylase, has the capacity of breaking interior chains (as well as end chains), that is, chains between anomalies, for no high molecular compounds similar to the β-dextrin are formed. On the other hand, the enzyme does not attack the 1,6-D-glucosidic linkages forming the branching points, for these are left unaffected in the limit dextrins. We must conclude that the malt α-amylase attacks normal maltose linkages. This is also shown by the fact, discussed later on, that it attacks amylose and short chain saccharides with maltose linkages. There remains to be explained the fact that about 17% of the linkages in starch are broken much more easily than the rest.

In the following experiment a thin paste of arrow-root starch was treated with malt α-amylase.[75] The degree of hydrolysis was ascertained by titration with hypoiodite and D-glucose and maltose were determined by fermentative methods. After the fermentation, the average chain lengths of the α-dextrins was determined by titration with hypoiodite before and after complete hydrolysis with sulfuric acid. In Fig. 4, curve I represents the relative number of D-glucosidic linkages ruptured by the enzyme, and curve II the average chain length of the α-dextrins formed (after the removal of fermentable sugar). In the dextrinization, the number average chain length of the products very rapidly decreases to about 8 and in the saccharification very slowly to about 5. Very prolonged action of the enzyme finally results in fermentable sugar and a mixture of dextrins with somewhat lower average molecular weight. These are the *limit dextrins.*

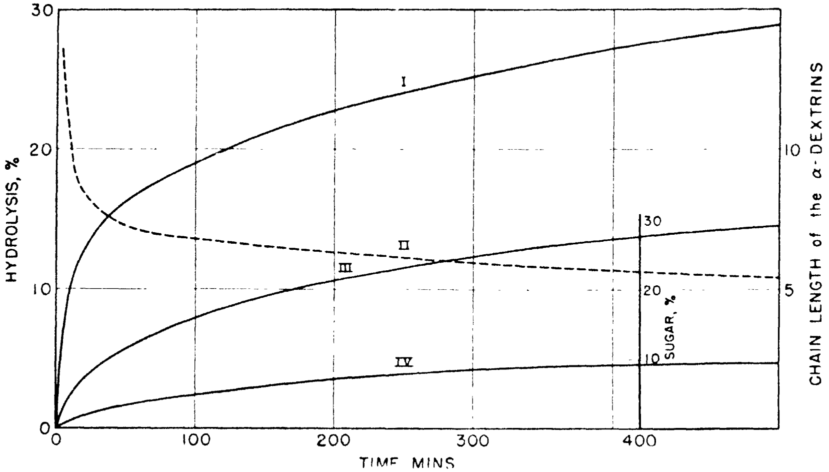

FIG. 4.—Hydrolysis of arrow-root starch by malt α-amylase. I, hydrolysis as calculated from reduction value; II, average chain length of α-dextrins; III, formation of maltose (and maltotriose); IV, formation of D-glucose.

a. Malt α-Amylase and Potato Starch.[76] The hydrolysis of potato starch paste gives a curve similar to that shown for corn starch (Fig. 3). Here likewise the dextrinization involves the opening of about 17% of the D-glucosidic linkages in the starch.

A thick paste containing 720 g. of starch in 9 liters of water was treated with malt α-amylase (until the mixture was only faintly colored

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(75) K. Myrbäck, *Biochem. Z.*, **311,** 227 (1942).

(76) B. Örtenblad and K. Myrbäck, *Biochem. Z.*, **307,** 123 (1941).

by iodine) and then boiled. Determination of the reducing power showed that 13% of the D-glucosidic linkages had been opened; hence, the dextrinization was incomplete. After removal of fermentable sugar (7.7%) by fermentation, the filtered solution was concentrated and the dextrins fractionated with increasing volumes of alcohol. In this way the dextrin preparations PDI to PDVIII (Table III) were isolated. The mother liquor from PDVIII was concentrated to a small volume and again precipitated with alcohol to give preparations PDIX to PDXIII. The mother liquor from PDXIII was concentrated to a thick sirup and dried with absolute alcohol to yield fraction PDXIV. This last fraction contains various impurities. Some properties of the fractions are shown in Table III.

TABLE III

α-Dextrins from Potato Starch and Malt α-Amylase at 13% Hydrolysis

Fraction	*Yield in per cent*	*Color with iodine*	$[\alpha]_D$	*Mol. wt.*	*P, %*	*Maltose, %*	
						β-Amylase method	*Salivary amylase method*
PDI	1.24	Violet	—	4100	0.267	35	—
PDII	19.02	Brown	196°	4100	0.143	38	49
PDIII	11.09	Red	202	2940	0.112	40	54
PDIV	4.64	Yellow	193	1910	0.129	53	63
PDV	12.14	None	191	1150	0.048	99	89
PDVI	3.11	"	187	1090	0.017	100	98
PDVII	0.39	"	184	1100	0.016	98	—
PDVIII	1.52	"	—	950	0.030	86	—
PDIX	11.01	"	189	1250	0.058	91	92
PDX	3.88	"	187	1150	0.013	100	99
PDXI	0.56	"	186	1070	0.022	104	—
PDXII	2.14	"	184	1020	0.014	100	100
PDXIII	2.29	"	182	1030	0.014	94	103
PDXIV	1.12	"	—	750	0.047	72	—

It is obvious that the fractions obtained in this manner are nonhomogeneous and must contain mixtures of chains of somewhat varying lengths. Fraction PDIX, which was precipitated after concentration of the mother liquor, would be expected to be rather nonhomogeneous. This fraction was examined by means of adsorption analysis by Tiselius and Hahn,[76a] and was shown to contain 19% pentasaccharide, 40% hexasaccharide and 38% of two higher saccharides, presumably hepta- and octasaccharides. The fraction PDXIII was investigated in the same

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(76*a*) A. Tiselius and L. Hahn, *Kolloid-Z.*, **105**, 177 (1943).

way. It consisted of 68% hexasaccharide, 19% pentasaccharide, 8% tetrasaccharide and 5% trisaccharide.

The saccharification of the α-dextrins with β-amylase was determined.[77] Table III shows that while the fractions PDI to PDIV are saccharified only partly, the fractions PDV to PDXIV give practically 100% maltose. These fractions correspond to 52% of the starch by weight, and if we take into consideration that about 20% of the starch substance was lost in the fractionation, washing and drying of the dextrins, it can be assumed that about 60% of the α-dextrins are completely saccharified by the β-amylase. This means that these fractions are *normal* α-dextrins which contain only 1,4-α-D-glucosidic linkages. In other words, these α-dextrins correspond to those parts of the starch that give 60% maltose in the direct action of β-amylase. The normal α-dextrins are, therefore, derived from the amylose and from the end chains of the amylopectin. The dextrin fractions PDI to PDIV, which are saccharified incompletely (*anomalous α-dextrins*) are derived from the branched nucleus of the amylopectin and contain the anomalies (branching points, 1,6-linkages).

The α-dextrin fractions, PDV to PDXIV, are completely saccharified also by salivary amylase.[78] The salivary amylase produces more maltose from the anomalous fractions of the α-dextrins than does β-amylase. The reason is, as pointed out above, that these fractions are not completely dextrinized. If we assume for example, that an α-dextrin of molecular weight 3400 contains two branching points (Fig. 5) only the part A-B is saccharified by β-amylase. But salivary amylase, being an α-amylase and capable of attacking the chains between branching points, splits the molecule, as at C, and then not only A-B but also C-D is transformed into maltose. When, after the action of the saliva on the Fraction PDII, the unfermentable dextrins were isolated, they were found to have an average degree of polymerization of 7. As the α-dextrin fraction PDII originally had the degree of polymerization of 25, it is clear that from every α-dextrin molecule an average of two limit dextrin molecules have been formed.

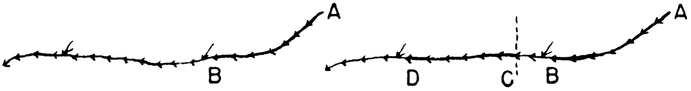

FIG. 5.—Hydrolysis of anomalous α-dextrin.

If the α-dextrins are treated for a long time with α-amylase they are saccharified more or less completely. This saccharification corresponds to the second slow action of the enzyme on starch. In contradistinction

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(77) K. Myrbäck, *Biochem. Z.*, **307**, 132 (1941).
(78) K. Myrbäck, *Biochem. Z.*, **311**, 242 (1942).

to the β-amylase and the salivary amylase, the malt α-amylase gives a slow saccharification of the α-dextrins without an absolutely definite limit of saccharification. Some of the α-dextrin preparations mentioned above were treated with α-amylase, and the production of maltose and D-glucose was measured by fermentation[79] (Table IV). As maltotriose is fermented by ordinary yeasts as well as maltose, it is probable that the sugar called "maltose" in Table IV is a mixture of maltose and maltotriose. In this case the saccharification would be more complete than appears from the last column of the table. It is evident that the normal α-dextrins (PDV to PDXII) are saccharified faster and more completely than the anomalous fraction PDII. The saccharification of the normal fractions in fact does not deviate very much from a monomolecular reaction. The saccharification curve of the fraction PDII, however, has another form. After a short time, the sugar production falls to a much lower value than initially. This means that in the first period there are

TABLE IV

Saccharification of α-Dextrins with Malt α-Amylase

Fraction	*Mol. wt.*	*Time of enzyme action*	*Sugar, %*		
			D-Glucose	*Maltose*	*Fermentable sugar as D-glucose*
PDII	4100	5 min.	2.5	5.5	8.3
		30 "	3.4	9.2	13.1
		2 hr.	5.5	19.7	26.3
		24 "	9.4	54.1	66.6
PDV	1150	5 min.	3.0	9.5	13.0
		30 "	5.5	16.0	22.4
		2 hr.	9.8	27.2	38.5
		24 "	14.0	68.1	86.0
PDVI	1090	30 min.	4.3	16.1	21.3
		60 "	5.9	25.0	32.3
		125 "	7.0	34.1	43.0
		27 hr.	13.5	70.8	88.2
PDIX	1250	30 min.	2.2	12.0	16.9
		60 "	5.6	16.9	23.4
		2 hr.	6.9	27.5	36.0
		24 "	10.7	68.1	82.7
PDXII	1020	30 min.	4.4	15.5	20.8
		60 "	5.3	23.3	29.9
		2 hr.	6.7	37.6	46.4
		27 "	13.1	70.9	87.9

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(79) B. Örtenblad and K. Myrbäck, *Biochem. Z.*, **315**, 233 (1943).

produced dextrins as well as fermentable sugar and these dextrins contain more "anomalies" and are thus saccharified slowly.

In some recent experiments[79a] the formation of fermentable sugars from a normal α-dextrin (hexasaccharide) by malt α-amylase was determined. The total yield of fermentable sugar was 100% and was found to be composed of 17% D-glucose, 60% maltose and 23% maltotriose. It is not improbable that maltotriose is converted to maltose and D-glucose by the enzyme, but the velocity of this transformation must be comparatively low.

Whereas β-amylase and salivary amylase split the α-dextrins and starch at much the same rate, the malt α-amylase has a much slower action on the α-dextrins (Table V).

TABLE V

Comparative Rate of Hydrolysis of Starch and α-Dextrin by Amylases

Enzyme	*Relative velocity of hydrolysis*	
	Starch	*Hexaose (α-dextrin, PDXII)*
β-Amylase	1	0.96
Salivary amylase	1	1.04
Malt α-amylase	1	0.17

In order to understand why the malt α-amylase acts so slowly on a normal hexaose (Fraction PDXII) we have tried to apply the Michaelis-Menten theory and have determined the affinity constants of the enzyme-substrate compounds.[80] If the concentration of starch and dextrin are expressed as moles of maltose per liter we find the affinity constant, K, between malt α-amylase and starch to be about 200 and for β-amylase about 170. The affinity constant α-amylase to α-dextrin is so small that no values could be obtained. The constant certainly is smaller than 12. Thus, it is clear that the slow action of the malt α-amylase on the dextrin is due to a very low affinity of the enzyme to the substrate. In other words, the enzyme can readily attach itself to a long chain, but only with difficulty to chains shorter than a certain value. In the dextrinization period of the action of the enzyme on starch, a mixture of α-dextrins is produced with an average chain length of about 7 units. It can be assumed, therefore, that the enzyme readily attaches itself to chains that contain more than about 8 units provided that the chains are normal and contain no branchings or other anomalies. When anomalies occur these

(79a) K. Myrbäck, *Arch. Biochem.*, **14**, 53 (1947).

(80) K. Myrbäck and N. O. Johansson, *Arkiv Kemi, Mineral. Geol.*, **20A**, No. 6 (1945).

will act just as end groups so that, for example, a chain containing 12 D-glucose units with a 1,6-linkage somewhere in the middle will be attacked only very slowly. Thus, the action of the malt α-amylase can be understood if we assume that the enzyme molecule, in order to attack a chain molecule without difficulty, must have access to a normally built part of the molecule having a length of at least 6 to 8 units.

If this conception of the α-amylase action is correct the enzyme should also have a slow action on products with short chains produced by the acid hydrolysis of starch. This is also the case. When hydrolyzed starch is treated with malt α-amylase the number of D-glucosidic linkages which are rapidly hydrolyzed by the enzyme ("dextrinization") decreases with increasing degree of hydrolysis[81] (Table VI; see also Section III on acid hydrolysis).

TABLE VI

Effect of Preliminary Degree of Acid Hydrolysis on Additional "Rapid" Hydrolysis by Malt α-Amylase

Average chain length of substrate	*Linkages split in the dextrinization phase, %*
Very high	17
8.5	5.3
5.5	1

b. Malt α-Amylase and Corn Starch.[82] A thin paste of corn starch was treated with the enzyme and the reduction calculated as degree of hydrolysis (Table VII). The curve of the plotted data has the same appearance as in the case of potato starch. At about 17% hydrolysis, the reaction velocity suddenly falls to a very low value. In one experiment with 600 g. of corn starch, the products of the enzyme action were fractionated when the number average chain length was 9. In this case, the dextrinization was not quite complete. The α-dextrin preparations MDI to MDX were obtained (Table VIII). In a second experiment, the fractionation took place when the average chain length of the products was 7.2. The fractions MDXI to MDXV (Table VIII) were obtained.

TABLE VII

Rate of Hydrolysis of Corn Starch by Malt α-Amylase

Time, min.	*Degree of hydrolysis*
10	11.7
30	16.5
50	18.5
70	19.1
120	19.3

(81) K. Myrbäck and Walborg Thorsell, *Svensk Kem. Tid.*, **55**, 178 (1943).

(82) K. Myrbäck and B. Örtenblad, *Biochem. Z.*, **316**, 429 (1944).

In this experiment 16% of fermentable sugar was formed. Although the fractions and especially those with the highest molecular weights assuredly are mixtures of saccharides with somewhat varying chain lengths, it can be stated that the enzyme, with corn starch as with potato starch, gives α-dextrins of which about 50% have chain lengths of about 7 whereas the other 50% have somewhat longer chains.

Experiments with fraction MDI and malt α-amylase showed that about 5% of the D-glucosidic linkages, that is, on the average one linkage in every dextrin molecule, is split very rapidly. This phase of the hydrolysis is a residue from the dextrinization phase. After this about 40% of the D-glucosidic linkages are split at the much lower velocity corresponding to the liberation of maltose and D-glucose.

TABLE VIII

α-Dextrins from Corn Starch by Action of Malt α-Amylase

Fraction	*Yield, %*	*Mol. wt.*	*Fraction*	*Yield, %*	*Mol. wt.*
MDI	37.5	3200	MDIX	0.7	1120
MDII	15.0	2900	MDX	2.8	940
MDIII	5.0	2450			
MDIV	4.2	1680	MDXI	2.8	
MDV	5.5	1190	MDXII	32.5	1800
MDVI	10.5	1180	MDXIII	14.0	1200
MDVII	2.2	1150	MDXIV	6.3	1070
MDVIII	1.0	1100	MDXV	11.8	880

c. Malt α-Amylase and Arrow-root Starch.[83] When the degree of hydrolysis is plotted against time, a curve is obtained which has just the same shape as in the case of potato and corn starch.

One experiment was stopped when the degree of hydrolysis was 13.2%. Fermentable sugar was removed. The average number chain length of the α-dextrins was 10. Fractionation with alcohol gave the dextrin preparations ADII to ADXII of Table IX (fraction ADI was very small and was not further investigated). Another experiment was stopped at 16.4% hydrolysis and the α-dextrin fractions ADXIII to ADXXII were isolated (Table IX).

The data of Table IX show that the composition of the α-dextrin mixture is nearly the same as in the case of the other starches investigated. Experiments with β-amylase indicated that the fractions with $M < 1300$ gave high yields of maltose and that the fractions with higher molecular weight gave only about 50% maltose. The results are the same as for potato and corn starch. Experiments with malt α-amylase

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(83) K. Myrbäck and Barbro Martelius, *Biochem. Z.*, **316**, 414 (1944).

showed that the α-dextrins from arrow-root starch also behaved like the dextrins from potato starch.

TABLE IX

α-Dextrins from Arrow-root Starch

Fraction	*Yield, %*	[α]D	*Mol. wt.*	*Per cent maltose with β-amylase*	*Fraction*	*Yield, %*	[α]D	*Mol. wt.*	*Per cent maltose with β-amylase*
ADII	26.9	184°	3400	53.8	ADXIII	0.5	—	—	—
ADIII	14.5	184	3150	—	ADXIV	3.7	188°	2450	46
ADIV	12.5	184	3000	50.6	ADXV	10.7	189	2400	40
ADV	3.4	185	2500	52.9	ADXVI	8.4	189	2200	43
ADVI	12.3	185	1750	71.2	ADXVII	7.8	189	2100	48
ADVII	10.3	181	1300	84.6	ADXVIII	8.8	189	1900	77
ADVIII	4.9	177	1300	83.5	ADXIX	16.0	184	1450	—
ADIX	2.6	180	1250	88.1	ADXX	19.6	178	1150	77
ADX	2.2	178	1200	—	ADXXI	9.4	177	1100	89
ADXI	0.7	179	1200	—	ADXXII	6.1	174	800	82
ADXII	3.7	180	1100	—					

d. Malt α-Amylase and Barley Starch.[84,85] The curves representing the degree of hydrolysis and the average chain length of the α-dextrins are practically the same as for the other starches mentioned above. In three experiments the reaction was stopped at different degrees of hydrolysis (10.1, 15.1 and 18.3%). The distribution of the dextrins of different chain lengths is shown in Fig. 6. The bottom curve represents the true α-dextrins from barley starch. As in the case of the other starches investigated about 50% of the α-dextrins have a chain length of approximately 7. These are the normal α-dextrins and they are almost completely saccharified by the β-amylase. The other 50% with chain lengths 8 to 13 are anomalous and are saccharified to only 40 to 60%.

e. Action of Malt α-Amylase on Amylose and on β-Dextrin.[54] In the following experiments two preparations of corn amylose, prepared according to Meyer, were used. The preparations were completely saccharified by β-amylase and should therefore contain only normal, unbranched molecules. For comparison, experiments were conducted with corn starch and β-dextrin from corn starch (Table X). The corresponding curves are found in Fig. 3. All curves are of the same type, which means that the reaction in all three cases consists of a dextrinization and of a saccharification phase. But the number of D-glucosidic linkages split in the dextrinization period is different: for amylose it is 22%; for starch

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(84) K. Myrbäck and G. Stenlid, *Svensk Kem. Tid.*, **54**, 103 (1942).

(85) K. Myrbäck, G. Stenlid and Gunhild Nycander, *Biochem. Z.*, **316**, 433 (1944).

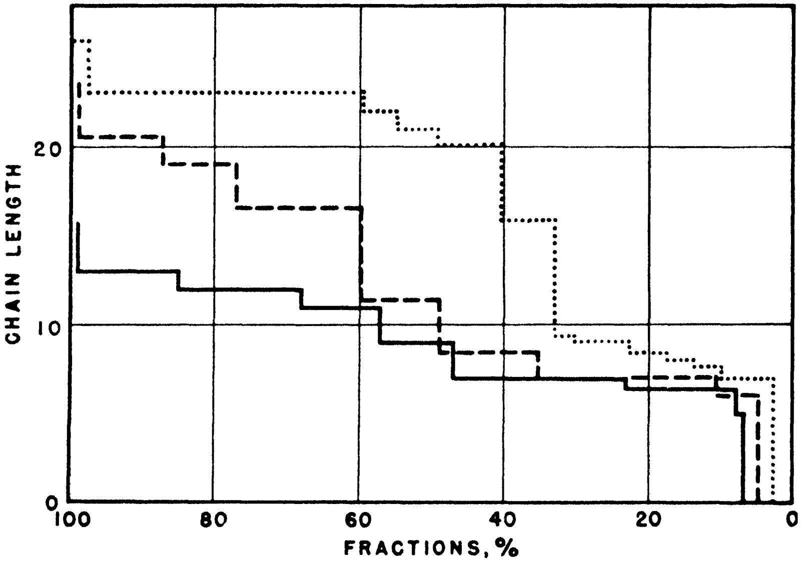

FIG. 6.—Dextrinization of barley starch; chain length distribution.

TABLE X

Action of Malt α-Amylase on Amylose, Starch and β-Dextrin

Time	*Amylose*				*Corn starch*		*β-Dextrin*	
	Prep. I	*Prep. II*						
	Hydrolysis, %	*Hydrolysis, %*	*Maltose, %*	D-*Glucose, %*	*Hydrolysis, %*	*Fermentable sugar, %*	*Hydrolysis, %*	*Fermentable sugar, %*
2 min.	—	5.4	3.3	<1	—	—	—	—
3 "	—	—	—	—	—	—	0.7	0
6 "	11.1	—	—	—	2.3	0	1.6	—
9 "	13.2	15.0	6.0	<1	3.2	0	—	—
15 "	17.5	18.5	—	—	5.1	3.1	3.1	<2
30 "	22.2	22.1	13.6	<1	8.7	5.7	5.1	4.4
60 "	—	23.0	17.2	—	11.5	14.6	7.1	6.1
120 "	—	24.2	—	—	14.7	18.9	9.1	8.2
12 hr.	—	31.6	33.4	3.5	—	—	—	—
24 "	40.0	—	—	—	26.4	43.0	10.4	—
4 days	—	45.5	82.0	—	35.5	—	11.3	13.0
8 "	46.5	48.4	—	—	39.5	—	11.5	14.0
32 "	—	53.5	91.8	8.6	43.5	—	—	—

17%; and for β-dextrin about 10%. It is clear that this is due to the different degrees of ramification of the substrates.

In an experiment with 8.5 g. of amylose and malt α-amylase, the enzyme action was stopped when the degree of hydrolysis was 22.4%, that is, at the end of the dextrinization phase. Fermentable sugar was removed by fermentation (17%) and the non-fermentable α-dextrins were fractionated with alcohol. Six fractions were obtained (Table XI) having chain lengths from 4 to 10 units.

In order to explain the action of the malt α-amylase, the assumption had been made formerly (Freudenberg, Meyer) that the enzyme has the capacity of attacking *all* D-glucosidic linkages in a chain, except the two terminal ones, with about the same probability. If this be the case, the distribution of the products at a certain degree α of hydrolysis can be calculated approximately according to the formula

$$F_n = n\alpha^2(1 - \alpha)^{n-1}$$

(Kuhn,[86] Sillén[87]) where F_n is the yield of saccharides with n units. In the above experiment 70% of the amylose was recovered as α-dextrins; 17% was removed as fermentable sugar (maltose, no D-glucose but probably maltotriose). About 13% was lost in the fractionation owing to the unfavorable nature of the precipitates. As the losses ought to be much the same for all fractions, we have recalculated the yields of α-dextrins (column 5, Table XI). If the yields of the saccharides with the chain lengths in question are calculated with the formula given above, we find the values in column 6. It is evident that the calculated values are quite different from the values found. Whereas practically no products with $n > 7$ are formed, the calculated yield is not less than 45%. Also, 53.5% of the amylose appears as α-dextrins with $n = 6$ to 7, whereas a yield of only 16% is calculated.

It was stated above that the action of malt α-amylase on starch is understandable if we assume that the enzyme must have access to a normal chain with at least 8 units. If a long chain molecule, such as the amylose molecule, is normal throughout, then all of its D-glucosidic linkages must be attacked with about the same probability with the exception of about three linkages at either end of the chain. These will be somewhat protected against the enzyme action. It is possible that the hindrance to the action may begin at the fourth linkage from the end group, grow stronger at the third and second and perhaps be complete at the first. As a first approximation, Sillén and Myrbäck[88] have assumed

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(86) W. Kuhn, *Ber.*, **63**, 1503 (1930).

(87) L. G. Sillén, *Svensk Kem. Tid.*, **55**, 221 (1943).

(88) L. G. Sillén and K. Myrbäck, *Svensk Kem. Tid.*, **56**, 142 (1944).

that three linkages at each end of every chain are attacked with a lower velocity than the other linkages. If the chain is very short, probably both end groups will have an influence on one and the same linkage. If the chain is rather long, it is probable that the linkage first hydrolyzed would lie at a good distance from the ends. If the chain has, for example, 10 D-glucose units, the linkage most easily hydrolyzed by the malt α-amylase will be in the middle of the chain. Then it is understandable that a very long, normal chain would eventually yield a mixture of α-dextrins in which those with 6 to 7 units would predominate.[30]

Table XI

α-Dextrins from Amylose

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Fraction	*Mol. wt.*	*n (D.P.)*	*Yield, %*		*Calcd. yield, %*				
			Found	*Corr. for loss*	*From Kuhn's formula*	*By assumption*			
						6 + 0	*5 + 1*	*4 + 2*	*3 + 3*
I	>1650	>10	0.4	0.5	28.1	0	0	0	0
II	1650	9–10	2.2	2.6	11.3	0	0	0	0
		8	0	0	6.8	0	0	0	0
III	1180	7	18.5	21.9	7.6	60.4	26.3	19.5	17.9
IV	1010	6	26.7	31.6	8.3	5.6	39.5	23.5	20.7
V	690	4–5	22.0	26.1	17.6	12.2	14.6	42.9	61.4
		2–3	17.2	17.2	15.7	14.0	19.7	14.2	0
		1	0	0	4.6	7.9	0	0	0
α			0.224			0.261	0.220	0.205	0.201

If we take into consideration that chain molecules of the amylose type have two end groups of different kind, it is possible that the number of terminal linkages which are protected against the enzyme action are not 3 + 3 as assumed above but perhaps 4 + 2 or 5 + 1 or 6 + 0. Sillén and Myrbäck[88] have attempted to calculate the distribution of the products under these assumptions (columns 7 to 10, Table XI). The assumptions "5 + 1" and "4 + 2" give values which are in fair agreement with the experimental data. As the values found for α and the yield of fermentable sugar should be the most accurate, the assumption "5 + 1" seems to be the best. This means that five linkages from one end group (possibly the non-reducing) and one linkage at the other end group are protected. It is, however, freely admitted that the fractionation of the α-dextrins is probably not sufficiently accurate to yield a definitive answer at the present time.

(The formation of D-glucose in the later stages of the reaction, that is, in the saccharification, shows that at least one terminal linkage can be attacked, but the velocity of the D-glucose formation is very low compared to the velocity of the dextrinization.)

If the enzyme attacks a branched molecule, it seems probable that the branching points and other anomalies act in the same way as the free end groups. The end chains of the amylopectin will be attacked as far as possible from the end group and the branching point and correspondingly the interior chains will be attacked somewhere in the middle. If the chain is very short, it will be hydrolyzed at a low velocity or perhaps not at all.

TABLE XII

Action of Malt α-Amylase on β-Dextrin

Time, min.	*Color with iodine*	*Hydrolysis, %*	D-*Glucose, %*	*Maltose + maltotriose, %*	*α-Dextrins*	
					Yield, %	*Chain length*
2	Violet	1.1	0	2	98	22
5	Red	3.0	0	3	98	15.5
10	Brown	5.4	0	5	96	12.7
15	"	7.3	0	6	95	12.3
24	?	9.4	0	6	95	10.9
36	None	11.5	0	8	94	10.2
50	"	11.5	0	8	90	10.1
70	"	11.5	0	8	90	9.6
100	"	12.1	1	9	90	9.5
145	"	14.4	2	11	88	9.2
200	"	14.4	2	12	84	8.7
45 hr.	"	34.4	5	52	45	5.7

In the β-dextrin all end chains have been removed by the β-amylase. Thus the action of the malt α-amylase only strikes the interior chains. The β-dextrin corresponds to 40% of the starch and has a degree of ramification of about 0.11. The interior chains have, according to Myrbäck and Sillén,[28] an average length of 10 units. It is possible that in the dextrinization period the longer interior chains will be ruptured more than once, but the very short chains will probably not be split at all, and, on the average, every chain will be split once. The experimental results are in good agreement with this view inasmuch as in the β-dextrin about 10% of the D-glucosidic linkages are split in the dextrinization. In another experiment (Table XII) the value 11.5% was found.[75] In this experiment the yields of maltose and D-glucose were determined as well as the average chain length of the α-dextrins produced (after removal of the fermentable sugars). The data of Table XII show that the yield of fermentable sugar is very low in the dextrinization period; this is due to the fact that the β-dextrin yields, almost exclusively, anomalous α-dextrins (n = about 10) with low saccharification velocity. Only after long times of action are appreciable amounts of fermentable sugars liberated, and then the chain length of the dextrins diminishes from 10 to about 6. After very prolonged action of the enzyme, more sugar is formed and the chain length of the dextrins diminishes further. In this phase of the reaction the abnormal α-dextrins are turned into limit dextrins.

TABLE XIII
Action of Malt Enzymes on β-Dextrin

Time, min.	*β-Amylase*		*α-Amylase*		*α-Amylase + β-amylase*	
	Color with iodine	*Fermentable sugar, %*	*Color with iodine*	*Fermentable sugar, %*	*Color with iodine*	*Fermentable sugar, %*
3	Violet	0	Violet	1.3	Violet	2.8
5	"	0	"	2.2	"	5.2
7	"	0	Red	3.0	Red	7.5
14	"	2	"	4.2	"	12.0
40	"	3	None	8.3	None	18.1
250	"	3.9	"	13.9	"	22.8
1400	"	—	"	18.6	"	29.0

When a β-dextrin is broken up by the α-amylase into α-dextrins, the α-dextrins, in contradistinction to the β-dextrin itself, are attacked by the β-amylase with the liberation of fermentable sugar[49] (Table XIII). This means that when maltose linkages in the interior chains of the β-dextrin are broken by the α-amylase, anomalous α-dextrins are formed, the normal end chains of which are saccharified by the β-enzyme (see Fig. 5, page 276).

If the above mentioned ideas regarding the mechanism of the α-amylase action are substantially correct, we must assume that in the dextrinization of amylopectin (and glycogen), end chains with less than 6 units are not attacked but that end chains with more than 7 units yield normal α-dextrins with 5 to 6 units (as in the case of amylose) and new end chains of approximately 3 units. Interior chains with less than 7 units are split once; the shortest chains with one or two units not at all. Interior chains with $n > 11$ may be hydrolyzed in such a way that new end chains (with an average of 3 units) are formed in addition to normal α-dextrins of about 5 units. If the amylopectin is built up as supposed by Myrbäck and Sillén, it can be calculated[30] that in the dextrinization 5.5% of the linkages in interior chains, or 14.3% of the linkages in interior *and* end chains, are split (the percentages are based upon the total number of linkages in the starch). A total of 53% of the D-glucose units should appear in the end chains, and thus the molecular weight of the abnormal α-dextrins should be approximately 2050. As we have seen, the complete dextrinization of starch yields abnormal α-dextrins with molecular weights of this order of magnitude.

If we assume that a certain starch contains 20% amylose, we can calculate that $0.8 \times 14.3 + 0.2 \times 22 = 16\%$ of all linkages will be easily hydrolyzed and that $0.8 \times 53 + 0.2 \times 100 = 62\%$ of the starch should appear in normal α-dextrins. These values agree well with the values found.

f. Action of Malt α-Amylase on Glycogen.[30] The hydrolysis of glycogen by malt α-amylase is also divided into a dextrinization and a saccharification phase. In the first phase about 10% of the D-glucosidic linkages are ruptured. The curve is almost the same as for β-dextrin (Fig. 3, page 271). The rate of the dextrinization is lower for glycogen than for starch and amylose. This is due to the absence of the amylose component and to the fact that the chains of the glycogen are shorter than those of the amylopectin. If we try to apply the above mode of calculation we find that about 11.5% of the linkages in the glycogen should be split in the dextrinization, which value is in agreement with that found.

g. Summary. The action of the malt α-amylase on different starches, on amylose, β-dextrin and glycogen can be understood if we assume: (a) that the polysaccharides in question are built according to the pattern discussed in Section I; and (b) that the enzyme has a high affinity for normal chains longer than about 7 units but a very much lower affinity for short chains. In this respect, anomalies in chain molecules (such as points of branching) act as end groups. The action of the enzyme on starches from potato, corn, arrow-root and barley is practically the same.

3. *Action of "Malt Amylase"*

"Malt amylase" is a mixture of α- and β-amylase and has the following action on starch.

(1) The α-amylase breaks up the molecules into normal and anomalous α-dextrins.

(2) The normal α-dextrins are completely saccharified by both enzymes to give maltose and small amounts of D-glucose. From α-dextrins with an uneven number of D-glucose units the β-amylase yields

certain amounts of maltotriose. This sugar is also formed by the action of malt α-amylase on all α-dextrins.[79a,88a]

(3) The anomalous α-dextrins are partly saccharified by both enzymes into fermentable sugars, leaving those parts of the molecules which contain the anomalies as *limit dextrins.*

If we assume that the anomalies of the starch molecules are 1,6-D-glucosidic linkages (and phosphate linkages, see below), the limit dextrins should contain one (or possibly in some cases more than one) 1,6-D-glucosidic linkage somewhere in the middle of the molecule together with a certain number of normal maltose linkages. The latter are attacked by the enzymes not at all or only very slowly because of the presence of the adjacent anomalous linkage. It may be that limit dextrins are of the unbranched type (A in Fig. 7) but probably some of them have a point of branching (B in Fig. 7). In the few cases in which the constitution of a limit dextrin has been established, it has been found to be of type A.

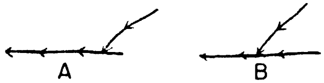

FIG. 7.—Unbranched and branched dextrin molecules.

We will discuss first whether there is an absolutely definite limit of action for all amylases. In the case of the action of β-amylase on starch and on α-dextrins this question seems to be settled, but in the case of the malt α-amylase the answer is less certain. But certainly the action of the "malt amylase" *practically* stops at a certain limit. There is, however, almost always a very slow further action. It is possible that this slow saccharification of the limit dextrins is due not to the amylases but to other carbohydrases which have no action on starch but which are capable of attacking products with short chains. Under all circumstances it must be kept in mind that when in an experiment the saccharification for practical purposes has stopped and the limit dextrins have been isolated, this does not necessarily mean that the limit dextrins will not be further attacked by the enzyme used. But the velocity of this action is certainly very small compared with the velocity of the action on starch. Thus, it must be admitted that experiments involving the isolation of the limit dextrins after the action of a certain amylase on starch are in most cases not strictly reproducible. The total yield and chain length distribution of limit dextrins may vary, but their general character is not affected. If a limit dextrin produced by a certain amylase is treated with the same enzyme for a very long time, it is very often transformed to another limit dextrin of lower molecular weight with concomi-

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(88a) K. Myrbäck and R. Lundén, *Arkiv Kemi, Mineral. Geol.*, **23A**, No. 7 (1946).

tant liberation of fermentable sugar. But this slow hydrolysis occurs mainly in the normal maltose linkages and the anomaly present in the parent limit dextrin is generally carried over into the second. In some cases the anomalous linkages are finally ruptured and the limit dextrin is then completely saccharified (as with Taka-amylase, see Section II, 4). That most amylase preparations have a certain action also on the 1,6-D-glucosidic linkages can be shown by experiments with isomaltose. The sirupy isomaltose of E. Fischer, prepared by "reversion" from D-glucose in strong hydrochloric acid, contains principally 6-α-D-glucosyl-D-glucose.[89,90] Experiments[51] show that this isomaltose was slowly hydrolyzed by Taka-amylase, pancreatic amylase and malt α-amylase. The action of saliva was very slight. Related reversion products, presumably also containing isomaltose linkages, were also attacked. Thus it seems reasonable that the limit dextrins would be attacked likewise although the rate of hydrolysis is extremely small.

TABLE XIV
Limit Dextrins from Corn Starch by Action of Malt Extract

Fraction	*Yield, %*	$[\alpha]_D$	*Mol. wt.*	
			By reduction	*By diffusion*
MMI	0.2	—	1650	—
MMII	2.7	172°	900	1160
MMIII	3.2	168	830	700
MMIV	2.3	162	680	650
MMV	9.5	157	580	525
MMVI	0.8	134	570	500
MMVII	1.2	137	520	470
MMVIII	0.5	—	—	—
MMIX	2.1	124	480	495
Total	22.5			

a. Nature of Limit Dextrins. From these considerations it follows that, for the present, it is impossible to speak of the limit dextrins as definite mixtures of saccharides derived from a certain starch by the action of a certain amylase. The only possibility is to allow the enzyme to act until no further action can be observed in a reasonable time and then to isolate the products. In the following experiments pastes of different starches were treated in this way with malt amylase (malt extracts or enzyme preparations purified by precipitation with tannin).

(89) K. Myrbäck, *Svensk Kem. Tid.*, **53**, 67 (1941).
(90) K. Myrbäck, *Svensk Kem. Tid.*, **53**, 264 (1941).

Fermentable sugars were removed by fermentation with bakers' yeast and the limit dextrins were fractionally precipitated with dilute alcohol.

b. Limit Dextrins from Corn Starch.[91] In this experiment, 1800 g. of corn starch in 20 liters of water was hydrolyzed with 200 ml. of malt extract prepared by extracting one part of ground malt for ten minutes with 2 parts of water containing toluene. The reaction was stopped when the rapid increase in reduction had ceased. The slow secondary saccharification of the limit dextrins therefore was not considerable and the yield of limit dextrins was high (Table XIV).

TABLE XV

Subfractionation of Limit Dextrin (MMV) from Corn Starch

Fraction	*Yield, g.*	*Mol. wt.*	
		By reduction	*By diffusion*
MMV1	0.5	—	—
MMV2	20.0	600	575
MMV3	23.3	600	625
MMV4	18.1	600	560
MMV5	6.2	600	650
MMV6	35.0	540	450
MMV7	12.3	510	500
MMV8	4.8	540	540
MMV9	21.1	540	540

These limit dextrins from corn starch contain saccharides with 3 to about 6 D-glucose units. About 50% seems to be trisaccharides. Fraction MMV (165 g.) was dissolved in water, treated with yeast to remove traces of fermentable sugar, and then fractionated a second time with alcohol. Nine fractions were obtained (Table XV). Total hydrolysis with mineral acid showed that the fractions contained about 5% impurities (from the malt extract). Therefore the values for the molecular weights show that the four last fractions contain only trisaccharides. Adsorption analysis[76a] showed, for example, that the fraction MMV8 contains a trisaccharide exclusively. The first four fractions are mixtures of tri- and tetrasaccharides, possibly with small amounts of higher saccharides. Clearly the trisaccharides can not contain maltose linkages exclusively. The maltotriose is, as mentioned before, fermented by bakers' yeast with great velocity, whereas the limit dextrins are only slowly fermented if at all.[44] Hence, it must be concluded that they contain at least one D-glucosidic linkage of another type. This is shown by

(91) B. Örtenblad and K. Myrbäck, *Biochem. Z.*, **303**, 335 (1940).

hydrolysis experiments with acids[92] (Table XVI). All limit dextrin fractions are hydrolyzed at a much lower rate than are maltose and starch. The monomolecular reaction coefficients are constant in the case of maltose, slightly increasing in the case of starch, and distinctly decreasing with the limit dextrins. Thus, the latter must contain linkages which are hydrolyzed more slowly than maltose linkages. This is in accordance with the assumption that 1,6-linkages occur in the limit dextrins, for isomaltose is hydrolyzed much more slowly than is maltose. The rate of hydrolysis of the limit dextrins decreases with decreasing chain length of the dextrin fraction and indicates that the number of normal maltose linkages is relatively greater in the dextrin fractions with longer chains. The simplest assumption is that all or nearly all limit dextrins contain one isomaltose linkage together with a varying number of maltose linkages.

TABLE XVI

Acid Hydrolysis of Limit Dextrin, $k \times 10^3$ in 2.34 N Hydrochloric Acid at 100°

Time, min.	*Maltose*	*Starch*	*Limit dextrin preparation*				
			MMII	*MMIII*	*MMIV*	*MMVI*	*MMIX*
20	7.80	4.20	3.34	3.17	3.26	3.25	3.21
60	7.78	4.62	3.48	3.43	3.50	2.87	2.85
90	7.66	4.77	3.45	3.35	3.28	2.81	2.83
200	7.83	4.4	3.2	3.06	3.00	2.6	2.5
330	—	—	2.81	2.70	2.68	2.36	2.2
500	—	—	2.1	2.5	2.3	2.1	1.9

	Limit dextrin preparation						
	MMV2	*MMV3*	*MMV4*	*MMV5*	*MMV6*	*MMV8*	*MMV9*
20	3.3	—	3.7	3.2	2.9	3.26	3.10
60	3.05	2.93	3.5	3.15	2.90	3.15	3.27
90	2.80	2.88	2.89	3.02	2.88	3.01	3.1
200	2.6	2.6	2.47	2.7	2.56	2.7	2.64
330	2.34	2.26	2.3	2.37	2.19	2.4	2.25
500	2.0	2.1	1.96	2.0	1.9	2.1	2.0

The specific rotation of the limit dextrins is lower than that calculated under the assumption that only maltose linkages occur. Maltotriose should have $[\alpha]_D$ about +153°.

The dextrin fraction MMIX was methylated. Vacuum distillation of the methylated product gave in good yield a sirupy methylated trisaccharide.[93] After hydrolysis (which was considerably slower than that of octamethylmaltose) one mole of tetramethyl-D-glucopyranose and two moles of sirupy trimethyl-D-glucose were isolated. Thus the trisaccharide contains an unbranched chain. Further it was shown that 50%

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[92] K. Myrbäck, B. Örtenblad and K. Ahlborg, *Biochem. Z.*, **307**, 53 (1940).

TABLE XVII

Limit Dextrins from Several Starches and Malt Extract

Fraction	*Yield, %*	*Mol. wt.*	
		By reduction	*By diffusion*
Rice starch			
RMI	0.49	1160	1380
RMII	11.60	1220	1460
RMIII	3.10	1100	1200
RMIV	1.36	820	1020
RMV	0.81	800	720
RMVI	0.68	685	630
RMVII	0.37	700	650
RMVIII	0.84	400	370
Total	19.25%		
Arrow-root starch			
AMI	0.15	—	—
AMII	0.25	2100	2400
AMIII	0.28	1360	1090
AMIV	3.82	760	660
AMV	0.11	650	—
Total	4.61%		
Barley starch + malt extract			
I	0.39	—	—
II	1.21	1060	—
III	4.94	640	—
IV	0.40	500	—
V	1.06	480	—
VI	0.05	370	—
Total	8.05%		

Fraction	*Yield, %*	*Mol. wt.*	
		By reduction	*By diffusion*
Wheat starch			
WMI	0.20	2600	2800
WMII	0.81	1330	1660
WMIII	1.13	960	930
WMIV	1.07	750	720
WMV	1.09	770	750
WMVI	0.67	690	710
WMVII	0.21	670	605
WMVIII	0.58	540	590
WMIX	0.36	470	430
WMX	0.17	440	400
Total	6.29%		
Barley starch + malt amylase			
I	3.63	2300	—
II	2.22	1900	—
III	0.90	1550	—
IV	0.33	1350	—
V	0.49	1150	—
VI	0.52	1000	—
VII	0.73	850	—
VIII	1.01	660	—
Total	9.83%		

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(93) K. Myrbäck and K. Ahlborg, *Biochem. Z.*, **307**, 69 (1940).

of the trimethyl-D-glucose fraction had a free primary alcohol group, and hence is presumably 2,3,4-trimethyl-D-glucose. Therefore this limit dextrin contains one maltose and one isomaltose linkage.

c. Limit Dextrins from Rice Starch.[94] 1800 g. of rice starch in 19 liters of water was hydrolyzed for eight weeks with 125 ml. of malt extract prepared by extracting malt with 5 parts of water containing toluene for six hours. The dextrin fractions are listed in Table XVII. The limit dextrins in this case have a chain length of 6 to 7 units. Considering the circumstances mentioned above, this cannot be taken as an indication that the limit dextrins from rice starch are essentially different from those derived from corn starch.

d. Limit Dextrins from Wheat Starch.[94] Wheat starch (1350 g.) in 10 liters of water was hydrolyzed for eighteen weeks with 100 ml. of malt extract. A strong secondary hydrolysis of the limit dextrins has occurred (Table XVII) and the total yield is low (6.3%). The limit dextrins isolated have chain lengths from 3 to 6 units but higher saccharides are also present.

e. Limit Dextrins from Arrow-root Starch.[95] Among the limit dextrins isolated (Table XVII) tetrasaccharides are predominant. The total yield is low owing to strong secondary saccharification of the primary limit dextrins.

f. Limit Dextrins from Barley Starch.[96] In one experiment, barley starch paste was treated with malt extract, in a second experiment with purified malt amylase (Table XVII). The purified enzyme has yielded limit dextrins with chain lengths greater than those given by the malt extract, possibly because certain carbohydrases capable of attacking the limit dextrins have been removed in the purification or because of a lower stability of the purified enzyme.

g. Limit Dextrins from Potato Starch.[94] In one experiment, nine fractions of the limit dextrins were isolated (Table XVIII). More than 75% of the total yield (14%) are tetrasaccharides. These are almost free from phosphoric acid. The limit dextrin fractions with higher chain lengths have a very high phosphorus content. The substitution with phosphoric acid impedes the action of the enzymes (see Section II, 9).

In a second experiment with potato starch, the hydrolysis was carried further so that only 6% of the starch was recovered as limit dextrins. About one-third of these dextrins were trisaccharides.

h. Summary. From these experiments with malt amylase and differ-

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(94) K. Myrbäck, B. Örtenblad and K. Ahlborg, *Biochem. Z.*, **315**, 240 (1943).

(95) K. Myrbäck, B. Örtenblad and Walborg Thorsell, *Biochem. Z.*, **316**, 424 (1944)

(96) K. Myrbäck, K. Ahlborg and B. Örtenblad, *Biochem. Z.*, **316**, 444 (1944)

ent starches it is evident that the "malt amylase" gives mixtures of limit dextrins. The latter usually have chains of 3 to 7 D-glucose units but may have longer chains of 10 to 12 units. The yield and composition of the limit dextrins are dependent on the purity of the enzyme preparation and on the time of enzyme action. It is not possible as yet to state the true composition of the residual dextrin mixture obtained by the action of pure α- and β-amylase from malt. It is clear that all limit dextrin fractions isolated have an abnormal constitution and are not built up exclusively of maltose linkages. The anomalies in the starch (amylopectin) molecules that are the cause of the limit dextrin formation are 1,6-D-glucosidic linkages, presumably identical with ramification points, and to some degree phosphate ester groups. It is not excluded that other kinds of anomalies may occur, but, as a whole, the production of the limit dextrins is very well explained on the assumption of branched molecules of the type discussed in Section I.

TABLE XVIII
Limit Dextrins from Potato Starch

Fraction	*Yield, %*	$[\alpha]_D$	*Mol. wt.*		*P, %*
			By reduction	*By diffusion*	
PMIX	0.11	—	—	—	0.50
PMX	0.55	—	1320	(750)	3.34
PMXI	1.09	—	1130	(860)	1.25
PMXII	1.06	157°	930	(695)	1.26
PMXIII	0.79	161	825	780	1.12
PMXIV	0.51	157	720	685	0.80
PMXV	8.72	146	575	—	0.055
PMXVI	0.83	140	600	525	0.050
PMXVII	0.36	145	575	460	0.035

If the 1,6-linkages (points of branching) represent the anomalies which cause the formation of the limit dextrins, it might be thought that all limit dextrins should have the same number of D-glucose units with the anomaly in the middle. But this is by no means a necessary inference. When the interior chains of the amylopectin are hydrolyzed by the α-amylase we obtain anomalous α-dextrins in which the chain carrying the reducing end group probably contains from one to about 5 D-glucose units. This chain is saccharified very slowly if at all by the amylases and remains in the limit dextrin formed from the anomalous α-dextrin when the two chains with non-reducing end groups are saccharified. Therefore it is apparent that the limit dextrins must have varying chain lengths.

4. *Action of Taka-amylase**

The action of the Taka-amylase seems to be much of the same type as that of malt amylase. The Taka-enzyme is an α-amylase; during its action, the viscosity of a starch paste rapidly diminishes, and after a short time the reaction mixture is not colored by iodine. In contrast to the malt amylase, the Taka-enzyme contains, as far as is presently ascertained, only one amylase. Its action seems to be of the same kind as that of the animal amylases. Taka-amylase and the animal amylases differ in the author's opinion from the malt α-amylase. They produce a dextrinization of the starch and subsequently a more or less complete saccharification of the α-dextrins, but the velocity of the saccharification is great compared to that of the dextrinization. This probably means that the difference in affinity of the enzyme for long and for short chains is not so pronounced as in the case of the malt α-amylase. In other words, the molecules of the Taka-amylase and the animal enzymes do not require as long pieces of normal chains for their attachment as the malt α-amylase. Therefore, the curve representing the hydrolysis of starch by Taka-amylase has no break like that in the curve for malt α-amylase, and in most cases it is impossible to isolate the unaltered α-dextrins. That α-dextrins of the same kind as those formed by the malt α-amylase can be produced by other amylases is shown by the fact that Waldschmidt-Leitz and Reichel[97] were able to isolate fairly large amounts of hexaoses after incomplete action of the pancreatic amylase. It seems to the author that we have reason to believe that the action of all α-amylases is essentially the same. Therefore, the same kind of limit dextrins should be expected. Differences may be due to the reasons discussed above, that is, to occurrence of other enzymes capable of splitting the limit dextrins. It is also possible that certain amylases during the saccharification of the anomalous α-dextrins may attack maltose linkages nearer to the anomalous linkage than certain other amylases; hence, limit dextrins of different chain lengths will be obtained, but their general nature should be the same. As mentioned before, the malt α-amylase differs from the other α-amylases at least in one respect: it produces D-glucose as a primary product.

a. Action on Corn Starch. In one experiment, 19.66% of the starch was isolated as limit dextrins[98] (Table XIX). About two-thirds of the dextrins had a chain length of 6; tetra- and trisaccharides were also present. The fraction MTIX (23 g.) was dissolved and refractionated (Table XX). The last two fractions contain trisaccharides.

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* An enzyme preparation known as Takadiastase and made from the mold *Aspergillus oryzae.*

(97) E. Waldschmidt-Leitz and M. Reichel, *Z. physiol. Chem.*, **233**, 76 (1934).

(98) K. Ahlborg and K. Myrbäck, *Biochem. Z.*, **297**, 172 (1938).

The fractions MTII to MTVI were dissolved in water and treated with Taka-amylase (230 g. in 2500 ml. of water + toluene + 2.5 g. of Taka-diastase). After two months about 75% of the D-glucosidic linkages were ruptured. Fermentable sugar was removed and the dextrins were fractionated after concentration of the filtrate (Table XXI). Fractions III and IV contained amorphous disaccharides (non-fermentable or slowly fermentable). Fraction III (8 g.) was methylated; distillation of the methylated product *in vacuo* gave a product with 54.2% methoxyl (calculated 54.6% for a methylated disaccharide). This substance (3.10 g.) after hydrolysis yielded 1.64 g. of tetramethyl-D-glucopyranose

TABLE XIX

Limit Dextrins from Corn Starch with Taka-amylase

Fraction	*Yield, %*	$[\alpha]_D$	*Mol. wt.*	
			By reduction	*By diffusion*
MTI	0.25	161°	865	810
MTII	4.95	184	865	1000
MTIII	6.75	179	895	940
MTIV	1.75	182	775	800
MTV	1.38	179	755	760
MTVI	1.48	164	715	700
MTVII	0.62	170	680	610
MTVIII	0.48	160	575	600
MTIX	2.00	150	480	480
Total	19.66%			

TABLE XX

Subfractionation of Limit Dextrin MTIX (See Table XIX)

Fraction	*Yield, g.*	$[\alpha]_D$	*Mol. wt.*	
			By reduction	*By diffusion*
MTIX1	0.2	—	—	—
MTIX2	2.4	153°	588	—
MTIX3	4.65	159	588	550
MTIX4	3.2	—	563	—
MTIX5	8.95	132	495	490
MTIX6	0.9	140	483	—

and 1.52 g. of trimethyl-D-glucose. The sirupy trimethyl sugar contained at least 70% 2,3,4-trimethyl-D-glucose; hence, the isolated disaccharide fraction contained chiefly isomaltose. The yield of disaccharide from the limit dextrins is in accordance with the assumption that every limit dextrin molecule contains one anomalous linkage.

TABLE XXI

Action of Taka-amylase on Limit Dextrins

Fraction	*Yield, g.*	*Mol. wt.*
I	18.5	550
II	12.2	465
III	21.5	362
IV	2.7	360

b. Action on Potato Starch.[94] Potato starch (1700 g.) in 14 liters of water was hydrolyzed with 1.3 g. of Taka-amylase. After ten days, the rapid increase of the reducing power ceased and only a very slow hydrolysis was observed. After twenty-seven days the mixture was boiled, treated with bakers' yeast, filtered and concentrated. Ten fractions of limit dextrins were isolated, Table XXII. Tetra- and hexasaccharides as usual are prominent, but longer chains are also present in considerable

TABLE XXII

Limit Dextrins from Different Starches with Taka-amylase

Fraction	*Yield, %*	*Mol. wt.*		*Fraction*	*Yield, %*	$[\alpha]_D$	*Mol. wt.*	
		By reduction	*By diffusion*				*By reduction*	*By diffusion*
	Potato starch				*Rice starch*			
PTI	0.23	—	—	RTI	0.05	—	—	—
PTII	0.94	3400	3000	RTII	10.40	170°	1070	—
PTIII	2.13	1860	1960	RTIII	7.15	170	810	940
PTIV	2.01	1730	1480	RTIV	0.36	173	1060	805
PTV	2.29	1380	1100	RTV	1.16	170	700	770
PTVI	1.55	1175	1080	RTVI	1.45	168	570	600
PTVII	0.51	1150	1030	RTVII	0.52	171	570	530
PTVIII1	3.34	830	810	RTVIII	0.12	155	540	525
PTVIII2		660	610	RTIX	0.65	—	(630)	430
PTIX	0.16	730	620					
Total	13.16%			Total	21.86%			

quantities. Prolonged action of Taka-amylase and other amylases on these limit dextrin preparations gradually gave increasing yields of fermentable sugar.

c. Action on Rice Starch.[94] Rice starch (1800 g.) in 15 liters of water was hydrolyzed with 1.5 g. of Taka-amylase for eight weeks (Table XXII). Chain lengths from 3 to about 7 units are represented. The hexasaccharide fraction is very large.

TABLE XXIII

Limit Dextrins from Arrow-root Starch with Taka-amylase

Fraction	*Yield, %*	$[\alpha]_D$	*Mol. wt.*		*P, %*
			By reduction	*By diffusion*	
ATI	0.2	—	—	—	4.89
ATII	2.2	170°	1320	1290	0.074
ATIII	10.7	165	670	660	0.026
ATIV	2.5	169	780	800	0.021
ATV	2.5	164	680	700	0.006
ATVI	0.1	—	—	—	Trace
ATVII	4.4	—	550	610	"
ATVIII	1.8	—	560	500	"
ATIX	3.7	—	500	475	"
Total	28.1%				

d. Action on Arrow-root Starch.[95] As seen from Table XXIII, the dextrin mixture contains tetra- and trisaccharides in large amount. The total yield of limit dextrins is high owing to a relatively short time of hydrolysis and a consequently low secondary conversion of the dextrins. Practically all combined phosphate appears in the smallest fraction with the highest molecular weight.

e. Action on Barley Starch.[96] The limit dextrins (Table XXIV) as before contain 3 to 7 D-glucose units. The small fraction with the highest molecular weight contains most of the combined phosphate.

5. *Action of Pancreatic Amylase*

Potato starch (800 g.) in 9 liters of water containing 2 g. of sodium chloride was hydrolyzed at 20° for twenty weeks with 1 g. of pancreatin (Pharm. Svec. Ed. X) (Table XXV). Apart from small amounts of higher saccharides, tetra- and trisaccharides predominate. The total yield of limit dextrins is high, possibly because the pancreatin contains lower amounts of dextrin-splitting enzymes or because its action ceases at a greater distance from the anomalies.

The rate of hydrolysis of the dextrin fractions in mineral acid was determined.[92] All fractions have a rate of hydrolysis lower than that of the starch and the reaction coefficient falls gradually. Surely this is due to the occurrence of the 1,6-D-glucosidic linkages.

Fraction PPX (15.8 g.) of Table XXV was treated with 2.85 N sulfuric acid at 100° for one hour. As a result, 57% of the D-glucosidic linkages were ruptured. Maltose and D-glucose were removed, and 5.2 g. of a sirupy disaccharide, fermented very slowly by bakers' yeast, was recovered. The rate of hydrolysis of this disaccharide in acid was considerably slower than that of fraction PPX; hence, the isomaltose linkages have been "concentrated" during the first hydrolysis.

TABLE XXIV

Limit Dextrins from Barley Starch with Taka-amylase

Fraction	*Yield, %*	$[\alpha]_D$	*Mol. wt.*	*P, %*
BTI	0.14	—	—	2.99
BTII	1.47	186°	1600	0.037
BTIII	3.14	186	1370	0.022
BTIV	4.44	185	1280	0.019
BTV	1.19	187	1010	0.046
BTVI	0.58	179	950	0.044
BTVII	0.81	169	730	0.058
BTVIII	0.46	—	745	0.120
BTIX	1.19	155	745	0.099
BTX	2.26	150	860	0.089
BTXI	0.71	165	800	0.112
BTXII	0.33	163	800	0.110
BTXIII	0.88	122	520	0.123
BTXIV	0.35	—	360	—
BTXV + BTXVI	1.66	—	350	0.365
Total	19.61%			

6. *Action of Salivary Amylase*[95]

Arrow-root starch (500 g.) in 8 liters of water containing 1 g. of sodium chloride was hydrolyzed at *p*H 6.5 with 50 ml. of saliva. After twenty-four hours, 50% of maltose had been formed. The reaction mixture was kept under toluene for thirty-two days. The reducing power then corresponded to 89% maltose. Fermentation experiments, however, showed that 63% maltose and 14% D-glucose had been formed, the latter by the secondary action of maltase. As mentioned above, the salivary amylase does not form D-glucose as a primary product but differs in this respect from malt α-amylase. The pancreatic enzyme is like the salivary amylase in this respect,[99] as in others. The limit dextrins formed in the above experiment were isolated as before (Table XXV) in a total yield of 24.1%. In another experiment, the hydrolysis was

TABLE XXV

Limit Dextrins Produced by Pancreatic and Salivary Amylases

Fraction	*Yield, %*	$[\alpha]_D$	*Mol. wt.*		*P, %*
			By reduction	*By diffusion*	
Pancreatic amylase on potato starch					
PPI	0.40	—	1120	—	3.44
PPII	0.25	—	1130	—	4.13
PPIII	3.45	162°	760	680	1.03
PPIV	2.12	167	730	600	0.551
PPV	2.02	166	710	650	0.165
PPVI	1.62	169	680	630	0.033
PPVII	8.31	148	630	545	0.055
PPVIII	2.56	155	620	560	Trace
PPIX	0.79	152	630	510	"
PPX	5.25	—	560	530	"
Total	26.77%				
Saliva on arrow-root starch					
ASI	0.2		4400	—	—
ASII	6.3		1680	—	—
ASIII	10.4		1050	—	—
ASIV	6.8		770	—	—
ASV	0.4		630	—	—
Total	24.1%				
ASVII	Trace		—	—	—
ASVIII	5.9		1870	—	—
ASIX	7.7		1500	—	—
ASX	3.1		1180	—	—
ASXI	2.0		800	—	—
ASXII	Trace				
Total	18.7%				

carried further and the yield was only 18.7% (Table XXV). The limit dextrins of salivary amylase seem to have somewhat longer chains than those produced by the other amylases. This may be due to the absence

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(99) K. Myrbäck, *Z. physiol. Chem.*, **158,** 160 (1926); **159,** 1 (1926).

of other enzymes with an action on the limit dextrins or to qualities of the amylase itself. As pointed out above it is possible that with certain amylases, the anomalies exercise their influence at a greater distance than with others.

When acted upon by large amounts of amylases, the limit dextrins mentioned above are hydrolyzed further. Taka-amylase, for example, gave after prolonged action nearly 100% D-glucose.

It was mentioned above that salivary amylase hydrolyzes normal α-dextrins, for instance, a malto-hexaose (and the corresponding acid) at practically the same velocity as it hydrolyzes starch.[58] This means that the affinity of the enzyme for long and for short chains is about the same. Consequently, the curve for the hydrolysis by salivary amylase has not the peculiar form of the curve for malt α-amylase but is continuous and has no sharp break. The α-dextrins which are formed at first are saccharified very rapidly. The normal α-dextrins are saccharified completely. The anomalous α-dextrins form the limit dextrins.

7. *Action of Bacterial Amylase*

Hopkins and coworkers[99a] have compared the action of malt α-amylase with that of bacterial amylase (from *B. subtilis*). The bacterial enzyme closely resembled the malt enzyme in respect to the kinetics of the reaction and produced much the same products.

8. *Summary of Action of Amylases on Starch*

Our experiments give the following picture of the enzymic degradation of starch by malt, Taka-, pancreatic and salivary amylases.

(1) The only action of the enzymes is the rupture of D-glucosidic linkages. The values of the molecular weight of the dextrins formed, determined in diffusion experiments and calculated from the reduction values, always agree so closely that we can assume that all products formed by these amylases are open-chain saccharides (containing, for example, no products of the type of the cycloamyloses).

(2) The β-amylase starts from the non-reducing end group and attacks chains with maltose linkages. Only maltose is liberated. Anomalies in the constitution of the chain molecule, such as isomaltose linkages, stop the enzyme action. The length of the chain plays no significant role. Maltotriose and maltose are not attacked. The enzyme is an "exo amylase."

(3) The α-amylases are "endo amylases," that is they have the capacity for attacking interior linkages in chains irrespective of their

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(99*a*) R. H. Hopkins and D. Kulka, *J. Inst. Brewing*, **48**, 170 (1942); R. H. Hopkins, D. E. Dolby and E. G. Stopher, *ibid.*, **48**, 174 (1942).

distance from end groups or anomalies, provided that the distance is not smaller than a certain value. Only normal maltose linkages are attacked.

(4) The malt α-amylase has a much higher affinity for long chains than for chains shorter than about 8 D-glucose members. Therefore, in long chains, a certain number of linkages are ruptured at a much higher velocity than the others. In very long normal chains, as in amylose, this number is about 22%. In a straight-chain saccharide, about 6 terminal linkages are more or less protected against the enzyme action, possibly in such a way that 5 linkages at one end and one linkage at the other are attacked slowly. On the other hand, the malt α-amylase can attack, even if slowly, at least one terminal linkage of a longer chain, thereby causing the liberation of D-glucose as a primary product. Anomalies in the chain molecules as, for instance, branching points, act on the malt α-amylase in much the same way as end groups. Therefore, in the branched molecule of the amylopectin, one linkage in every interior chain is ruptured on the average by the enzyme with about the same velocity as the 22% of the linkages in the amylose. This means that in ordinary starches the dextrinization caused by the enzyme involves about 17% of the linkages.

(5) In general, the other α-amylases have the same effect as the malt α-amylase, but the difference in affinity for long and for short chains is less marked. Thus, when the degree of hydrolysis is plotted against time, uniformly curved lines are obtained, whereas the malt α-amylase gives a sharp break at about 17% hydrolysis. The animal amylases do not yield D-glucose.

(6) The amylases investigated either do not attack the 1,6-D-glucosidic linkages or they attack them only with an extremely low velocity. Normal maltose linkages in the vicinity of the anomalous linkage are attacked with a velocity which decreases as the distance from the anomaly decreases. Therefore, the limit dextrins contain one or possibly sometimes more than one 1,6-D-glucosidic linkage together with a varying number (generally 1 to 5) of normal maltose linkages. This number may be somewhat different for different amylases and is gradually diminished as the time of action of the enzyme increases. It is possible, although in view of recent experiments not probable, that the slow saccharification is an action of other enzymes present in the amylase preparations.

9. *Phosphoric Acid in Starch*

Most starches contain phosphoric acid. In some cases (wheat starch) the phosphoric acid can be removed by extraction with suitable solvents[100]; in other cases the acid evidently is chemically united with the

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(100) T. J. Schoch, *J. Am. Chem. Soc.*, **64**, 2954 (1942).

starch or with some component of the starch. This must be true at least for potato starch. Posternak[101] has shown that by acid hydrolysis of potato starch a D-glucose phosphate is produced which is identical with the D-glucose 6-phosphate isolated by Robison and King.[102]

As some fractions of the limit dextrins produced by the common amylases have a much higher phosphorus content than starch, the author assumed[8,9] that the substitution with phosphoric acid is one of the anomalies postulated as the cause of the limit dextrin formation. That the phosphoric acid acts in this way is evident from the investigations of Posternak,[103] who isolated a tetraose phosphate from a mixture of limit dextrins. On acid hydrolysis this gave D-glucose 6-phosphate.

The phosphoric acid is not evenly distributed over the whole starch substance; amylose is free or almost free from phosphorus.[4,14] It may be mentioned that "amylose" prepared according to Ling is reported to have a very low phosphorus content. The author has obtained such preparations in some cases, but for the most part preparations were obtained which had the same or a higher phosphorus content than the starch.[9] These preparations were not completely saccharified by β-amylase and obviously contained amylopectin even if the solutions had a low viscosity.

Most amylases have only a slight action on the phosphate groups. As mentioned before, the β-dextrin from potato starch contains all the phosphorus of the starch. This is in accordance with the fact that the phosphorus is concentrated in the amylopectin. A β-dextrin from arrow-root starch had a phosphorus content of 0.051% while the starch itself had 0.019%. Since the β-dextrin corresponds to 40% of the starch, it is evident that here also all the phosphorus is left in the β-dextrin.

When potato starch is dextrinized by the malt α-amylase, all phosphorus of the starch is recovered in the α-dextrins[61] (Table III). It is evident that the phosphoric acid is concentrated in the fractions of α-dextrins with the highest molecular weight (anomalous α-dextrins). In fact, the four first fractions contain more than 76% of the phosphoric acid of the potato starch. The fraction PDV, which probably also contains molecules of a higher degree of polymerization, and the fraction PDIX, which was precipitated after concentration of the mother liquor, have a somewhat higher phosphorus content than the fractions with the average molecular weight 1050. Now, as mentioned before, it has been concluded from entirely other reasons that the first fractions with a molecular weight considerably higher than 1100 are derived from the

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(101) T. Posternak, *Compt. rend.*, **198**, 506 (1934).

(102) R. Robison and E. King, *Biochem. J.*, **25**, 323 (1931).

(103) T. Posternak, *Helv. Chim. Acta*, **18**, 1351 (1935).

interior chains of the amylopectin and contain the anomalous D-glucosidic linkages of the starch. Thus one is tempted to conclude that the phosphoric acid radicals in the amylopectin are situated in the vicinity of the points of branching. Synthetic amylose contains little or no phosphoric acid and the phosphoric acid remains on carbon atoms 1 or 4.[4] Thus it seems possible that the phosphoric acid in the 6-position in the potato starch has something to do with the formation of the 1,6-linkages. On the other hand, it is evident that the fact that the phosphoric acid in the α-dextrins is found in the fractions with the highest molecular weight does not prove that the phosphate groups are situated very close to the branching points. If, for instance, there is a phosphate group in an end chain of the amylopectin at a distance of four D-glucose units from the branching point, it is very possible that the α-amylase cannot attack the chain between the two anomalies.

The anomalous α-dextrins containing much combined phosphoric acid are only partly saccharified by β-amylase. Probably the substitution plays a role in this respect. But the phosphorus content is so low that its influence must be very small compared to the influence of the anomalous D-glucosidic linkages.

The limit dextrins always contain phosphorus and this is concentrated in the fractions of longest chain length. The limit dextrins obtained from potato starch with pancreatic amylase contain all the phosphoric acid of the starch (Table XXV). The two small Fractions PPI and PPII have a phosphorus content corresponding to one phosphate group in a saccharide with about five D-glucose units. The average degree of polymerization of the fractions was 6 to 7. Thus, if the phosphoric acid is evenly distributed over all molecules in the fractions, every dextrin molecule will contain one or in some cases two phosphate radicals. It seems logical to assume that the presence of phosphoric acid is the cause of the higher degree of polymerization of these fractions. All fractions very likely contain one anomalous D-glucosidic linkage and in some cases an additional phosphate group. If the degree of polymerization of a limit dextrin containing one anomaly is assumed to be four, then it seems probable that a dextrin containing two anomalies should have a somewhat longer chain. Under all circumstances, it is evident that most phosphate groups in the potato starch are situated in close vicinity to the points of branching.

Even if a strong secondary conversion of the dextrins has occurred and the total yield of limit dextrins is low, these dextrins will contain a great deal of the phosphorus content of the original starch. This is demonstrated by the data of Table XVIII.

Taka-amylase contains phosphatase and will gradually set free the phosphorus of the limit dextrins. But if the action of the enzyme is not very prolonged, most of the combined phosphoric acid of the starch is found in the limit dextrins (Tables XXIII and XXIV).

Most amylase preparations have a certain phosphatase activity. Some of the preparations used in our experiments were tested on glycerophosphate (Table XXVI) at *p*H 5.3 and 30°. Parallel experiments with potato starch (Table XXVI) show that the action of the phosphatases on the combined phosphoric acid in starch or limit dextrins, respectively, is very slight compared to the action on glycerophosphate. It is not clear why this is so. Possibly the phosphoric acid in starch is doubly esterified, thus forming a cross linkage between two chains. Or, it may be possible that pyrophosphoric acid occurs in starch.

TABLE XXVI

Phosphatase Activity of the Amylase Preparations

Enzyme	*Phosphoric acid liberated, %*							
	From glycerophosphate				*From dextrins*			
Time, days	*2*	*20*	*40*	*60*	*1*	*10*	*50*	*95*
Pancreatin	0	0	0	0	0	0	0	0
Saliva	2	9	17	21	0	0	0	0
Malt extract	25	69	101	—	0	0	0	0
α-Amylase	3	17	25	36	0	2	2	2
β-Amylase	12	36	45	60	0	2	3	3
Taka-amylase	8	44	65	74	0	2	5	10

10. *The Cycloamyloses* (*Schardinger Dextrins*)

The nature of the Schardinger dextrins now seems to be settled.[4] They are cyclic compounds (cycloamyloses) containing about six D-glucose units and only 1,4-D-glucosidic linkages. The amylase of *Bacillus macerans,* therefore, must be capable not only of rupturing the chain molecules but also of combining into maltose linkages the free ends of the saccharides so formed. The mechanism of this peculiar action of the enzyme is not clearly understood, but it seems reasonable to assume that it is connected with a capacity of the enzyme to arrange the chains in helical coils in such a way that when a D-glucosidic linkage is ruptured, the free end of the chain is in close proximity to the other end required to complete the cycle. Perhaps the formation of the new maltose linkage may be compared to the "conjugated heterosidification" (transglucosidation) of Rabaté,[104] occurring when phenyl glucosides are decomposed by certain glucosidases in the presence of even very small amounts of methanol.

Amylose gives a greater yield of cycloamyloses than amylopectin. Kerr[4] obtained 70% of cycloamyloses from corn amylose and from amylopectin 44%. Cycloamyloses are formed also from glycogen. On the other hand the β-dextrin yields no cycloamyloses.[105] The conclusion is inescapable that the cycloamyloses are formed from the simple chains of the amylose and from the end chains of amylopectin and glycogen.

When the *Bacillus macerans* enzyme acts on starch paste the viscosity decreases rapidly to a value only slightly higher than the water value of the viscometer. This proves that the enzyme breaks up the starch molecules, leaving no high molecular limit dextrins of the type of β-dextrin. Thus, it must be concluded that the *Bacillus macerans* amylase not only converts the amylose and the end chains of the amylopectin to cycloamyloses but also splits the branched part of the amylopectin into dextrins of low molecular weight. The enzyme, therefore, must be able to attack chains between anomalies (points of branching). It must, at least in the first part of the reaction, act like an α-amylase. The author has also observed that as the viscosity decreases there is a small increase in the number of reducing groups, which may result from the formation of dextrins with open chains.[67] When the average number degree of polymerization calculated from the reducing groups is about 100, the cycloamyloses appear, as may be judged from the appearance of the crystals in the test of Tilden and Hudson[106] (see Table XXVII). In this stage of the reaction no fermentable sugar is formed. The relation between the decrease in viscosity and the increase in the number of reducing groups was approximately the same for *Bacillus macerans* amylase and for malt α-amylase. This suggests that the first stages in the degradation are about the same for both enzymes.

In the authors' experiments, the cycloamyloses by very prolonged action of the enzyme did vanish with the production of maltose. It is not clear if this is an action of the *Bacillus macerans* amylase or of another enzyme present in the bacillus. After a very long time the yield of fermentable sugar was about 60% of the starch.

The action of β-amylase on starch partially degraded by the *Bacillus macerans* amylase was determined (Table XXVII). The amount of maltose decreases with decreasing degree of polymerization. When the

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(104) J. Rabaté, "Heteroglykosidasen" *in* Bamann-Myrbäck (editors), "Die Methoden der Fermentforschung," G. Thieme Verlag, Leipzig, p. 1818 (1941); Academic Press, New York (1945).

(105) R. W. Kerr, *J. Am. Chem. Soc.*, **65**, 188 (1943).

(106) Evelyn B. Tilden and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 2900 (1939).

yield of cycloamyloses is at its maximum, about 30% maltose (calculated on starch) is formed from the other products. The maximum yield of cycloamyloses (precipitated by trichloroethylene) was 37% in this experiment. This figure is certainly low because of losses in the preparation. Starch gives 60% maltose with β-amylase. In the above experiment about 40% of cycloamyloses and 30% of maltose were formed. This result would signify that maltose and possibly small amounts of cycloamyloses are formed not only from the amylose and the "natural" end chains of the amylopectin, but also from the "new" end chains formed by rupture of the interior chains of the amylopectin.

TABLE XXVII
Action of Bacillus Macerans Amylase

Time, hr.	*Relative viscosity*	*Hydrolysis, %*	*Color with iodine*	*Crystals with iodine*	*Fermentable sugar, %*	*Maltose by β-amylase, %*	*Linkages ruptured in dextrimization, %*
0	5.10	0	Blue	None	0	56	18.5
0.4	3.89	0.02	"	None	0	59	19.0
3	1.72	0.13	"	None	0	65	19.0
9.5	1.30	0.40	"	Few	0	59	15.3
24	1.21	1.05	"	Many	0	52	14.2
48	1.14	2.4	Brown	Very many	1	40	11.1
96	1.12	4.6	None	" "	2	31	9.7
216	1.11	9.8	"	" "	9	22	4.5
456	1.11	19.6	"	Many	25	19	4.2
864	—	37.0	"	None	61	15	2.0
2060	—	—	"	None	60	—	—

When the products of the action of the *Bacillus macerans* amylase on starch are treated with α-amylase (Table XXVII), one observes as usual a dextrinization and a saccharification phase. Expressed as percentage of the total number present in starch, the number of D-glucosidic linkages ruptured in the dextrinization phase diminishes with increasing time of action of the *Bacillus macerans* enzyme. This does not mean that the linkages ruptured by the *Bacillus macerans* enzyme are the same as those which are easily ruptured by the malt α-amylase; instead the *Bacillus macerans* enzyme splits all chains which can be split by the malt enzyme and produces dextrins having a chain length so short that they are not further dextrinized by the malt enzyme. These results support the idea that the action of both enzymes on starch is substantially the same with the difference that the *Bacillus macerans* enzyme recombines the free ends of the normal α-dextrins that are produced.

III. THE ACID HYDROLYSIS OF STARCH

When dilute mineral acids act on very dilute solutions of starch, the only end product of the hydrolysis is D-glucose, if phosphoric acid and minor constituents are disregarded. If the concentrations of acid and starch are very high, higher saccharides are formed in addition. These may to some degree be remnants of starch molecules, but are mainly secondary products formed by "reversion" from D-glucose in presumably the same manner as those isolable after the action of concentrated hydrochloric acid on D-glucose alone.[69,70] The "amylobiose" of Pringsheim certainly is a reversion product, even if it must be remembered that small amounts of isomaltose can be obtained by hydrolysis of starch under such conditions that no reversion occurs.

If a dilute solution of starch is treated at a suitable temperature with dilute mineral acid and the degree of hydrolysis α is calculated from the number of reducing groups set free, we find that the values of

$$k_m = \frac{1}{t_2 - t_1} \ln \frac{1 - \alpha_1}{1 - \alpha_2}$$

first increase until α is about 0.8 and then decrease rapidly. This decrease is due to the occurrence of the 1,6-D-glucosidic linkages, which are hydrolyzed at a lower rate than the maltose linkages. The increase in the k_m values in the first phase of the hydrolysis is due to the fact, emphasized by Freudenberg,[2] that the velocity of hydrolysis of a certain D-glucosidic linkage is dependent not only on the nature of the linkage but also on its place in the chain. Thus, the velocity of hydrolysis varies with the degree of polymerization of a saccharide even if it contains only linkages of one and the same type. The mathematical treatment of the hydrolysis of a polysaccharide with two types of linkages with different velocities of hydrolysis is exceedingly complicated, especially if the two types of linkages are mixed at random as seems to be the case in starch. But if the influence of the few 1,6-linkages, noticeable only in the last stages of the hydrolysis, is disregarded, it can be shown that the acid hydrolysis of starch is best interpreted by the assumption that in all saccharides all D-glucosidic linkages are split with the same velocity constant k except one terminal linkage which is split with the greater constant $k(1 + p)$.[107]

The amounts of D-glucose and of maltose and maltotriose formed

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(107) K. Myrbäck and B. Magnusson, *Arkiv Kemi, Mineral. Geol.*, **20A**, No. 14 (1945).

during the hydrolysis can be estimated in fermentation experiments. The quantities found agree with the values calculated under the above assumption. The amounts of higher saccharides cannot be determined with any accuracy. We have tried to get some information about them by observing the action of β-amylase and malt α-amylase on the products of hydrolysis (Table XXVIII). If the chain molecules of starch did not contain any anomalous D-glucosidic linkages, all products of the acid hydrolysis would be completely saccharified by the β-amylase. But if the starch has branched molecules the hydrolysis must yield both branched and unbranched saccharides. The unbranched ones are completely saccharified to maltose or maltose and maltotriose by the β-amylase. The branched products will only be partly saccharified. The enzyme will saccharify only that part of the molecule (A in Fig. 8) enclosed in dotted lines. The saccharide B (Fig. 8) will probably not be attacked at all. Thus the acid hydrolysis must influence in two ways

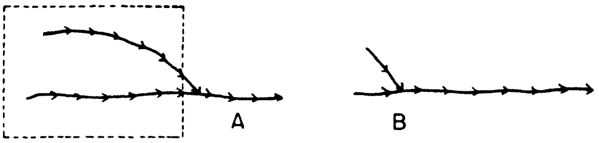

FIG. 8.—Action of β-amylase on branched dextrins.

TABLE XXVIII
Acid Hydrolysis of Starch

Acid hydrolysis			*Action of β-amylase*			*Action of malt α-amylase*
α, % (degree of hydrolysis)	*D-Glucose, %*	*Maltose + maltotriose, %*	*Linkages ruptured, %*	*D-Glucose, %*	*Maltose + maltotriose, %*	*Linkages ruptured in dextrinization, %*
0	0	0	31.5	0	61.0	16.2
4.3	0	0	33.1	0	63.0	12.2
6.9	0.5	0.1	35.5	0.5	63.5	11.4
10.0	1.4	1.5	36.7	2.0	62.0	9.2
21.0	5.6	12.4	40.3	5.3	61.0	5.4
34.7	13.0	25.2	46.9	12.6	55.5	3.1
63.7	38.0	38.0	67.1	40.0	39.5	0.8
80.5	57.0	30.2	81.0	61.5	34.0	0
92.0	85.0	11.5	92.0	81.0	12.5	0
96.4	91.0	7.2	95.8	91.2	8.0	0

the behavior of the substrate toward the β-amylase. First, new end chains are formed which can be attacked, and therefore the yield of maltose by action of the β-amylase is increased in the beginning of the acid hydrolysis. Secondly, as the hydrolysis proceeds, not only more D-glucose and maltose are formed but also products of Type B (Fig. 8), which are not saccharified. From the data of Table XXVIII it is seen that at $\alpha > 0.8$ (80%) the β-amylase has no action on the products. This shows that besides the fermentable sugar only products of Type B are present.

As mentioned before, the number of D-glucosidic linkages split by malt α-amylase in the dextrinization period decreases with the degree of acid hydrolysis and when α is about 0.6 (60%) there is no further dextrinization (Table XXVIII). This is due to the fact mentioned before that the enzyme attacks chains with less than about eight members only with very low velocity. When the number of chain molecules with longer chains is calculated for different degrees of hydrolysis, values for the extent of the dextrinization are found which agree fairly well with those found in the experiment.

IV. Bearing of Hydrolytic Studies on the Structure of Starch

We may conclude with the statement that not only do the experiments on the enzymic breakdown of starch point directly toward the irregular formula with multiple branchings, but that the results concerned with the acid hydrolysis are explicable on the same assumption.

THE POLYSACCHARIDES OF MYCOBACTERIUM TUBERCULOSIS[1]

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I. Introduction

The unique immunological and bacteriological properties of *Mycobacterium tuberculosis* have stimulated a great deal of research into the chemical composition of the cell and its associated substances. The initial impetus to these extensive investigations was given by Koch,[1a] who prepared various bacillary extracts and showed that these were able to stimulate specific reactions in infected animals.

The investigations of early workers[2,3,4,5] were directed into three main channels. Attempts were made first to isolate an "immunogen" from the substances of the cell or from the substances in the culture medium. A second line of research was the investigation of the nature of the skeletal

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(1) See M. Stacey, *Adv. Carbohydrate Chem.*, **2**, 162 (1946) and T. H. Evans and H. Hibbert, *ibid.*, **2**, 204 (1946) for the chemistry of other bacterial polysaccharides.

(1*a*) R. Koch, *Berlin. klin. Wochschr.*, **19**, 221 (1882); *Deut. med. Wochschr.* **16**, 1029 (1890); **17**, 101, 1189 (1891); **23**, 209 (1897); **27**, 829 (1901).

(2) A. Hammerschlag, *Z. klin. Med.*, **9**, 12 (1891).

(3) K. R. Hoffman, *Wien. klin. Wochschr.*, No. 38 (1894).

(4) E. A. de Schweinitz and M. Dorset, *J. Am. Chem. Soc.*, **17**, 605 (1895); **18**, 449 (1896); *J. Am. Med. Assoc.*, July 4th (1897).

(5) W. G. Ruppel, *Z. physiol. Chem.*, **26**, 218 (1898).

substances of the cell. In the third approach, attempts were made to determine the relation between the chemical composition of the bacilli and their virulence.

In each instance, polysaccharides were found to play a fundamental part. More recent workers have considered the polysaccharides from three sources: (*a*) those associated with the somatic portion of the cell; (*b*) those associated with the lipids; and (*c*) those associated with the "tuberculin" from the culture medium. The literature and variety of the results of these investigations is now so vast that in a review such as this, one cannot hope to give a complete survey of the field. The aim has rather been to arrive at a representative view of the work so far reported.

Comprehensive reviews of the earlier investigations have been published by Cornet[5a] and by Wells, Dewitt and Long.[5b] A recent review of the composition of tuberculin and the proteins of acid-fast bacilli has been published by Seibert.[6] Anderson[7] has summarized the chemical knowledge of the lipids of *M. tuberculosis.*

II. POLYSACCHARIDES OF SOMATIC ORIGIN

Extensive investigations, particularly of an analytical nature, were carried out on the composition of the cell membrane by Hammerschlag,[2] Hoffman,[3] de Schweinitz[4] and Ruppel.[5] Hammerschlag claimed that the "foundation" substance of the cell wall was cellulose, and Baudran,[8] using zinc chloroiodide, obtained a positive color reaction said to be characteristic of cellulose. The validity of these results was questioned in the light of later researches.[9] The cell wall of the organism has a highly complex structure. It does not contain cellulose and is not composed solely of lipid material, as was once thought.[9a]

The presence of pentoses in fractions of *M. tuberculosis* was first reported by Bendix.[10] Tamura[11] also obtained evidence for the presence

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(5*a*) G. Cornet, "Tuberculosis and Acute General Miliary Tuberculosis," W. B. Saunders and Co., Philadelphia, Pa. (1904).

(5*b*) H. G. Wells, Lydia M. Dewitt and E. R. Long, "The Chemistry of Tuberculosis," The Williams and Wilkins Co., Baltimore, Md. (1932).

(6) Florence B. Seibert, *Chem. Revs.*, **34**, 107 (1944).

(7) R. J. Anderson, *Chem. Revs.*, **29**, 225 (1941); *ibid. in* "Fortschritte der Chemie organische Naturstoffe," Julius Springer, Wien, Vol. 3, p. 145 (1939); *ibid.*, *Harvey Lectures*, Ser. 36, 271 (1939–1940); *ibid.*, *Yale J. Biol. Med.*, **15**, 311 (1943).

(8) G. Baudran, *Compt. rend.*, **142**, 657 (1906).

(9) R. J. Dubos, "The Bacterial Cell," Harvard University Press, Cambridge, Mass., p. 88 (1945).

(9*a*) T. Nishimura, *Arch. Hyg.*, **18**, 318 (1893).

(10) E. Bendix, *Deut. med. Wochschr.*, **27**, 180 (1901).

(11) S. Tamura, *Z. physiol. Chem.*, **89**, 304 (1914).

of pentoses in the cell but failed to detect cellulose. After the removal of fats and proteins from the cell by treatment with organic solvents followed by dilute alkaline extraction, a residue was obtained which was insoluble in alkali and in Schweitzer's reagent. This residue failed to give any coloration with iodine, thus excluding the possibility of the presence of glycogen. Tamura's product was readily hydrolyzed by dilute mineral acid. The hydrolyzate, which gave a positive orcinol test for pentose, yielded an osazone on treatment with phenylhydrazine. The properties of this osazone corresponded to those of arabinose phenylosazone. It was concluded that the initial fraction was a pentosan or a pentose-containing polysaccharide. No precautions seem to have been taken to fractionate the product or to remove nucleic acid. The latter substance itself gives a positive orcinol test for pentoses and on hydrolysis yields a sugar (D-ribose) which forms an osazone identical with that of D-arabinose. Tamura obtained only very small yields of his phenylosazone.

Panzer[12] obtained a gel-like carbohydrate substance from defatted bacilli (human strain) by successive extraction with water, hydrochloric acid, sodium carbonate, sodium nitrate, hot water and potassium hydroxide. The resulting liquors each yielded a gum-like substance which was sulfur-, nitrogen- and phosphorus-free. Oxidation of this derivative with nitric acid gave oxalic acid but no mucic acid. Panzer concluded that the substance was probably a pectin but not a galactan. It is now known that drastic oxidation of this nature would give oxalic acid from any sugar derivative. It is possible that by using controlled oxidation, mucic acid could be obtained from this carbohydrate.

Levene,[13] studying the relationship between the virulence and chemical composition of *M. tuberculosis*, isolated a glycogen-like substance by extraction of the bacilli with sodium chloride or with ammonium chloride. The extract was treated with picric acid in acetic acid and the resulting precipitate of nucleic acid removed. Cupric chloride was added to the filtrate and the carbohydrate-copper complex precipitated by the addition of alcohol. Purification was effected by repeated precipitation with alcohol from an acidified aqueous solution. The product was soluble in water and gave a coloration with iodine characteristic of glycogen. It was precipitated by basic lead acetate and was not reducing to Fehling solution until after hydrolysis by dilute mineral acid. Only traces of nitrogen and phosphorus were present.

Warkany[14] isolated two polysaccharide fractions from *M. tuberculosis;*

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(12) T. Panzer, *Z. physiol. Chem.*, **78**, 414 (1912).

(13) P. A. Levene, *N. Y. Med. Record,* Dec. 17th (1898); *J. Med. Research,* **6**, 135 (1901); **12**, 251 (1904).

(14) J. Warkany, *Z. Tuberk.*, **42**, 184 (1925).

one was resistant to acid hydrolysis and the other non-resistant. The acid-resistant substance was isolated by heating the defatted bacilli first with dilute sulfuric acid, then with potassium hydroxide. The residue, after being thoroughly washed with water and organic solvents, was free from readily hydrolyzable substances and fats. Without further investigation, this residue was designated "cellulose." The non-resistant polysaccharide fraction was obtained by prolonged heating of the bacilli with strong potassium hydroxide solution. The residue was removed by filtration and the polysaccharide precipitated from the filtrate with alcohol. This water-soluble product was reducing to Fehling solution only after hydrolysis with dilute acid, and was designated "glycogen." However, Warkany showed that pentoses were present in the hydrolysis liquor (furfural test) and that tests for chitin were negative. This work is now open to some doubt. The products claimed were named without rigorous chemical proof and the homogeneity of the products was not proven. The yields of products obtained (7.1% cellulose, 4.1% glycogen, 4.15% pentose) took no account of ash content. Precipitation of "glycogen" from a strongly alkaline extract of cells would lead to the coprecipitation of inorganic substances, particularly phosphates.

Zinsser and Parker,[15] and Zinsser and Tamiya,[16] isolated carbohydrate material from autoclaved pulverized bacilli by extraction with saline solution at *p*H 9.9 for four hours. Treatment of the extract with 10% acetic acid precipitated a nucleoprotein fraction. The supernatant liquor was made alkaline with sodium hydroxide and the solid matter, largely phosphate, was separated. No coagulable protein was obtained on boiling the filtrate with acetic acid. The solution was adjusted to *p*H 7, and a further quantity of phosphate was removed. This solution, it was claimed, contained the "residual antigen." The solution was biologically active and contained alcohol-insoluble substances, although the antigenic potency was not quantitatively correlated with fractions obtained by graded alcoholic precipitation. It was suggested that this "residual antigen" was of a polysaccharide character and represented a "haptophore" on which the whole specificity depended. The active principle was not able to stimulate antibody formation. The chemical nature of the substance was not investigated further. It was found, however, that it was heat-stable and retained its activity after contact with alkaline and acid solutions for protracted periods.

In 1925, Laidlaw and Dudley[17] completed investigations concerning the somatic polysaccharide constituents of *M. tuberculosis*. Their

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(15) H. Zinsser and J. Parker, *J. Exptl. Med.*, **37**, 275 (1923).

(16) H. Zinsser and T. Tamiya, *J. Exptl. Med.*, **42**, 311 (1925).

(17) P. P. Laidlaw and H. W. Dudley, *Brit. J. Exptl. Path.*, **6**, 197 (1925).

results provided a starting point for later extensive researches. They isolated the polysaccharides by extracting fat-free bacilli with mild warm alkali (2% potassium hydroxide and 2% potassium acetate). "Tuberculinic acid," the nucleic acid of *M. tuberculosis*, was removed from the acidified extract in the form of the copper salt. Any remaining tuberculinic acid and protein was adsorbed on uranium phosphate. The biologically active polysaccharide fraction was coprecipitated with uranium hydroxide. The uranium was removed by conversion to the phosphate and the polysaccharide was precipitated from the filtrate by alcohol. After purification, it was found that the polysaccharide contained two components. One of these was identified as glycogen. It was a white, water-soluble powder, imparting a characteristic brown color to iodine solutions. The coloration disappeared on heating and reappeared on cooling. The substance was readily hydrolyzed with dilute acid. A phenylosazone identical with that derived from D-glucose was isolated from the hydrolysis mixture.

The second polysaccharide fraction was a white solid which was soluble in water yielding a viscous solution which had $[\alpha]_D + 67°$. It gave no iodine coloration. Hydrolysis with dilute acid gave 50% reducing sugar (calculated as glucose), in which there was 30.6% pentose. Part of a phloroglucide obtained from the pentose was soluble in alcohol, this being interpreted as indicating the presence of a methylpentose in the polysaccharide. No crystalline derivatives were obtained from the hydrolyzate, nor could the presence of D-glucose be proved. This polysaccharide showed high serological activity in its reaction with the homologous serum of immunized animals in a dilution of 1:6,400,000. The substance, however, on injection into animals failed to elicit antibody formation, and it was therefore classified as a haptene.

Maxim[18] extracted a sample of dry fat-free bacilli with acidulated water. The extract was concentrated and the protein removed. The cell residue was treated with water at 80° and the extract removed as before. A third extract was made in alkaline solution by tryptic digestion. The purest product was obtained from the first extraction by precipitation with alcohol. Glycogen was removed from this product by repeated precipitation with alcohol and strong acetic acid. Polysaccharides were obtained from the second and third extracts by precipitation of the copper and uranium complexes in alkaline solution in the cold. A purified product was obtained by elimination of the copper and uranium.

This purified polysaccharide gave an almost colorless aqueous solution, a negative biuret test, no coloration with iodine and an intense Molisch

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(18) M. Maxim, *Biochem. Z.*, **223**, 404 (1930).

test. According to the method of extraction and degree of purity, typical data obtained were: ash, 1.4–0.03%; $[\alpha]_D$ + 64.5 to +80.2°; nitrogen 1.1–0.5%; hydrolysis (0.5 N sulfuric acid), $[\alpha]_D$ between −18° and −24°, giving 58–72% reducing sugar. The substance was serologically active and reacted with immune serum in a dilution of 1:3,000,000.

In 1932, Heidelberger and Menzel,[19] by extraction of *M. tuberculosis* (H37 strain (human strain 37) grown on Long's medium) with dilute acetic acid and fractional precipitation by alcohol, obtained a polysaccharide mixture. Glycogen was removed from this mixture by the enzymic action of saliva. Two specific polysaccharides were identified. One was characterized by its high optical rotation and yielded D-arabinose and D-mannose on hydrolysis; the other had a lower rotation and a greater solubility in water. D-Arabinose only was identified on hydrolysis of the latter polysaccharide, while D-mannose seemed to be absent. These observations, it was claimed, reconciled the work of Laidlaw and Dudley[17] with the results obtained by Mueller.[20,21] The first fraction contained 0.1% nitrogen, had a neutral equivalent of about 7,000, $[\alpha]_D + 85° \rightarrow +95°$, and on hydrolysis yielded 87% reducing sugar (D-arabinose and D-mannose were recognized) calculated as glucose. The precipitin titer with immune serum was 1:50,000. The second fraction contained between 0.6–0.8% nitrogen, had an equivalent weight of about 2,000 and $[\alpha]_D$ + 77°. Hydrolysis gave 61% reducing sugar, mainly D-arabinose, and the precipitin titer with immune serum was 1:50,000.

Gough,[22] also in 1932, attempted to identify the acidic components of the hydrolysis mixture of the polysaccharide of the tubercle bacillus. The polysaccharide was isolated by the action of 4% sodium hydroxide on the moist bacilli. When the major portion of the cells had passed into solution, the liquid was acidified and tuberculinic acid removed from the filtrate by precipitation as the copper salt. The filtrate was treated with hydrogen sulfide to remove excess copper, dialyzed and concentrated to a small volume. The product was obtained by precipitation with alcohol. Purification and removal of glycogen was effected by the method of Laidlaw and Dudley.[17] The product had an ash content of about 1%, a phosphorus content of 0.8% and contained fractions having $[\alpha]_D$ + 47° to +111°, which were strongly acidic. The precipitating power against immune serum was 1:3,000,000 to 1:5,000,000. A fraction having

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(19) M. Heidelberger and A. E. O. Menzel, *Proc. Soc. Exptl. Biol. Med.*, **29**, 631 (1932).

(20) J. H. Mueller, *J. Exptl. Med.*, **43**, 9 (1926).

(21) J. H. Mueller, *Proc. Soc. Exptl. Biol. Med.*, **22**, 209 (1925).

(22) A. L. C. Gough, *Biochem. J.*, **26**, 248 (1932).

$[\alpha]_{5461} + 52°$ was acetylated using pyridine and acetic anhydride. The acetylated derivative, which was insoluble in water, ether and light petroleum ether, showed $[\alpha]_{5461} + 42.9°$ in ethyl alcohol. On deacetylation with sodium hydroxide, a polysaccharide of almost identical $[\alpha]_{5461}$ and with the same serological activity as the initial substance, was regenerated. Gough showed that the polysaccharide was readily adsorbed on Lloyd's reagent.

Enzymic degradation experiments on the polysaccharide were unsuccessful. Hydrolysis was carried out with 2.5% sulfuric acid, and in the hydrolyzate, D-mannose was identified as the phenylhydrazone, D-arabinose as the benzylphenylhydrazone and D-galactose as the methylphenylhydrazone. The acidic components gave a negative naphthoresorcinol test for hexuronic acids, though indications of the presence of glycolic acid were claimed.

Ludewig and Anderson[23] isolated from *M. tuberculosis* (human strain) a purified polysaccharide fraction, which was essentially free from both phosphorus and nitrogen (0.7%) but contained 4% ash. It was isolated from an alcohol-ether extract of bacilli, by precipitation with lead acetate in ammonia. Hydrolysis of the polysaccharide gave a glucose equivalent (reducing sugar) of 65%, of which 27% was present as D-mannose; D-arabinose and D-glucose together represented 33%. The acetylated polysaccharide contained 49.3% acetyl and had $[\alpha]_D + 64°$. Inositol (the common meso form, see page 46) and an unidentified acid were also obtained from the products of hydrolysis.

Inositol, D-arabinose and D-mannose were identified as constituents of a polysaccharide obtained from human strain bacilli by du Mont and Anderson.[24] The substance was isolated by a method similar to that employed by Ludewig and Anderson.[23] The polysaccharide, which contained 11.9% ash and 1.27% nitrogen, was then acetylated giving a derivative containing 44% acetyl. Similar results were obtained by these authors for the polysaccharide isolated from the avian strain of bacilli.

In 1937, Heidelberger and Menzel,[25] published the results of an extension of their earlier investigations. The polysaccharide residue was isolated as before by prolonged extraction of fat-free bacilli (H37 strain) with acidulated water. Glycogen was removed with saliva and the remaining polysaccharide mixture was fractionated by various methods (methyl alcohol-water; acetic acid-water). It was found that the crude polysaccharide was a mixture of serologically active and inac-

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(23) S. Ludewig and R. J. Anderson, *Z. physiol. Chem.*, **211**, 103 (1932).

(24) H. du Mont and R. J. Anderson, *Z. physiol. Chem.*, **211**, 97 (1932).

(25) M. Heidelberger and A. E. O. Menzel, *J. Biol. Chem.*, **118**, 79 (1937).

tive fractions. Of the two principal active fractions, one had a relatively high dextrorotation, $[\alpha]_D + 79°$, and a low pentose content; in it D-arabinose and D-mannose were identified after hydrolysis. The other serologically distinct fraction had $[\alpha]_D + 24.2°$. On hydrolysis, D-arabinose only was obtained, there being no evidence to suggest the presence of D-mannose in this case. Both these fractions contained traces of nitrogen, ash and phosphorus; tests for inositol were negative.

The serologically inactive polysaccharide fractions were strongly acidic, and it seemed that the inactivity was in some measure related to the acidic function. These workers suggested that immunological specificity, in the case of the tubercle bacillus, was not connected with sugar acids. They also showed that the polysaccharide associated with the culture medium, as isolated by Masucci, McAlpine and Glenn,[26] contained constituents identical with those in polysaccharides of somatic origin.

The polysaccharide constituents of Bacillus Calmette-Guérin, were studied by Chargaff and Schaefer,[27] the method of isolation being similar to that employed by Heidelberger and Menzel.[25] Fat-free cells were extracted with an acetate buffer at *p*H 4.1 (in the presence of phenol) for a prolonged period. A fraction was obtained which gave a blue-violet coloration with iodine. This was in agreement with other workers who isolated such a substance under the name of "glycogen." The first serologically active polysaccharide had $[\alpha]_D + 77°$ and contained 0.8% nitrogen and 0.9% phosphorus. The fraction readily underwent hydrolysis with dilute acid, yielding 77% reducing sugar (calculated as glucose). D-Mannose (46%) and D-arabinose (8%) were identified, though neither D-galactose, uronic acid nor any other acidic components were found in the hydrolyzate. Traces of inositol and a small amount of D-glucosamine (2.9%) were present. It was suggested that the latter substances formed part of the non-reducing components of the polysaccharide. Both the crude and purified polysaccharide reacted with anti-(Bacillus Calmette-Guérin) serum in a dilution of 1:1,000,000. Injection of the substance into rabbits produced no polysaccharide anti-serum, an observation which added confirmation to the view that the polysaccharide functioned as a haptene only.

The second polysaccharide fraction isolated by Chargaff and Schaefer[27] was insoluble in alkali and in water; it contained 9.7% phosphorus, 47.7% ash and was nitrogen-free. On acid hydrolysis, 46.7% reducing sugar was obtained. It was shown that this fraction was an intimate mixture of

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(26) P. Masucci, K. L. McAlpine and J. T. Glenn, *Am. Rev. Tuberc.*, **22**, 669, 678 (1930).

(27) E. Chargaff and W. Schaefer, *J. Biol. Chem.*, **112**, 393 (1935).

polysaccharide material and calcium phosphate, which was separated by reprecipitation from ammoniacal solution by the addition of alcohol and a little glacial acetic acid. The purified polysaccharide then gave rise to 93.6% reducing sugar on hydrolysis and reacted with immune serum in dilutions from 1:1,000,000 to 1:2,500,000.

In 1939, Heidelberger and Menzel,[28] using their previous methods, isolated a polysaccharide mixture from *M. tuberculosis* (bovine strain). The mixture consisted of serologically active substances with a larger amount of inactive polysaccharides. The specificity of the active fractions was the same as that of the corresponding ones isolated from the human strain. Two such fractions were obtained. The two main inactive components had a high dextrorotation and contained traces of nitrogen and phosphorus, as well as a small amount of glycogen. The active fractions reacted with immune serum in dilutions of 1:4,000,000 to 1:2,000,000, respectively. Evidence was given that this serological activity of the bovine strain polysaccharides was related to the pentose content of the substances.

Karjala and Heidelberger[29] studied the polysaccharides of the avian strain of *M. tuberculosis.* The fat-free organisms were extracted with 0.35% acetic acid for a long period. The liquor was treated with glacial acetic acid, chloroform and uranyl acetate to remove colored impurities, protein and phosphates, respectively. The crude polysaccharide was fractionally precipitated from solution by organic solvents and yielded acidic fractions containing traces of nitrogen and phosphorus, and some fractions containing pentoses. The specific polysaccharide fractions, which were the fractions containing the greatest amount of pentoses, showed the same immunological specificities as those derived from the human and bovine strains. The active polysaccharide reacted with H37 anti-serum (serum corresponding to human strain bacilli). A smaller quantity of polysaccharide was obtained from the avian bacilli than from the human or bovine bacilli. No phosphorylated serologically inactive polysaccharide corresponding to that of the bovine bacilli was present in the avian strain.

The nature of the glycogen associated with *M. tuberculosis* has been investigated by Chargaff and Moore.[30] This substance was isolated from defatted bacilli by means of a borate buffer at *p*H 8.5 and separated as a jelly on supercentrifuging at low temperature. The dialyzed protein-free product was then lyophylized (concentrated to dryness by ice sublimation). The mother liquors contained mainly nucleoprotein. The

(28) M. Heidelberger and A. E. O. Menzel, *J. Biol. Chem.*, **127**, 221 (1939).

(29) S. A. Karjala and M. Heidelberger, *J. Biol. Chem.*, **137**, 189 (1941).

(30) E. Chargaff and D. H. Moore, *J. Biol. Chem.*, **155**, 493 (1944).

glycogen fraction, which gave a characteristic coloration with iodine, was also obtained by extraction of bacilli with trichloroacetic acid. The orcinol test, Seliwanoff test and the test for uronic acids were all negative. On hydrolysis with 1% hydrochloric acid at 100° for two hours, a reducing value of 102–108% was obtained. All the glycogen fractions were biologically inactive. It was noted that enzymic degradation with amylase proceeded more slowly than with liver glycogen. D-Glucose was identified in the hydrolyzate as the *p*-nitrophenylhydrazone and as the *p*-bromophenylhydrazone.

The physical properties of the glycogen were investigated by the Tiselius electrophoretic method and by the Svedberg ultracentrifuge. The following results were obtained.

Diffusion constant (D_o) = 0.925×10^{-7} (1% barbiturate buffer, *p*H 7.8, 1.5°).
Partial specific volume (V_{27}) = 0.63 (*c*, 0.5% in water).
Viscosity = 0.09 (*c*, 1.04% in water at 20.2°).

The particle weight (from sedimentation, diffusion and partial specific volume data) was calculated as 12.1×10^{-6}. The frictional ratio, f/f_o, 1.59, corresponded to an axial ratio of 11, the particle being depicted as an elongated ellipsoid. Similar measurements[31] with liver glycogen gave a particle weight of 13.2×10^{-6} (based on viscosity increment and diffusion data).

Carbohydrates have long been thought to be associated with the nucleic acid of the bacilli. Bendix[10] prepared from the intact cell a nucleoprotein fraction which, after hydrolysis, yielded derivatives which were reducing to Fehling solution and gave a green coloration in the orcinol test. Bendix thus claimed that pentoses were present in the nucleoprotein of *M. tuberculosis.*

The chemistry of "tuberculinic acid" (the nucleic acid of the tubercle bacillus) was investigated by Brown and Johnson.[32] The acid was purified by conversion to the copper salt. Distillation with hydrochloric acid yielded small amounts of furfural, indicating the presence of only a trace of pentose in the residue. Levulinic acid was identified, and it was thought on this evidence that the sugar associated with the acid was a hexose. Tuberculinic acid is unique in that it does not contain uracil, has a low pentose content and contains an unusual pyrimidine derivative. The tuberculinic acid was considered to be more nearly related to deoxyribonucleic acid than to ribonucleic acid.[32a]

(31) J. W. Mehl, J. L. Oncley and R. Simha, *Science,* **92,** 132 (1940).

(32) E. B. Brown and T. B. Johnson, *J. Am. Chem. Soc.,* **45,** 1823 (1923).

(32*a*) For a review of the chemistry of nucleic acids, see R. S. Tipson, *Advances in Carbohydrate Chem.,* **1,** 193 (1945).

The nucleic acid of the tubercle bacillus has been identified largely as deoxyribonucleic acid. This acid has been found to occur in association with polysaccharide fractions, probably due to the remarkable adsorptive powers of the latter. The nucleic acid, which is antigenically inactive (see p. 325), has been successfully separated from protein and carbohydrate material by electrophoretic means.[33]

III. POLYSACCHARIDES ASSOCIATED WITH TUBERCULIN AND THE CULTURE MEDIUM

Investigations concerning the nature of the biologically active substances present in the medium after growth of *M. tuberculosis* have led to the identification of polysaccharide constituents, though later it was shown by Seibert[6,34] that the substance reponsible for the tuberculin skin reaction was essentially a protein. Mueller[21] obtained a polysaccharide by fractional precipitation of the culture medium with alcohol. The product, which contained less than 1% nitrogen, behaved as a haptene (it could react with immune serum (1:1,000,000) but could not stimulate antibody formation). It was also very weak in promoting a skin reaction. Mueller[20] worked out a method for the isolation of this polysaccharide, which consisted essentially of an alcoholic precipitation of the medium, as before, followed by purification of the precipitate by dialysis; those components which were precipitated by acid or alkali, or formed copper complexes, were removed. The purified polysaccharide was a yellow gum having $[\alpha]_D + 24.2°$. It gave a mixture of reducing substances upon acid hydrolysis. The presence of pentoses was indicated by the formation of a phloroglucide; however, no red phloroglucide was obtained and this was taken to mean that 6-deoxyhexoses were absent. No crystalline derivatives were obtained from the hydrolyzate. The polysaccharide was found to have the following properties: nitrogen, 0.3%; phosphorus, trace; ash, 3%; reducing power (calculated as glucose), 99%. On hydrolysis, $[\alpha]_D$ changed to $-22.9°$ and the hydrolyzate contained 54% pentose. The polysaccharide gave a strong Molisch test and was not precipitated by any of the usual protein precipitants such as picric, tannic or phosphotungstic acids, basic lead acetate, copper sulfate, mercurous nitrate, or uranium nitrate. It possessed a fairly high degree of biological activity, in spite of the rigorous method of isolation.

Though no further steps were taken to prove its chemical homogeneity, it was claimed that this polysaccharide was of similar nature to that isolated by Laidlaw and Dudley[17] from the somatic portion of the

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(33) Florence B. Seibert, *J. Biol. Chem.*, **133**, 593 (1940).

(34) Florence B. Seibert, *Science*, **63**, 619 (1926).

cell. The biological properties of the polysaccharide obtained by Mueller were studied in more detail by Enders.[35] It was found that the substance could induce lethal anaphylactic shock in tuberculous guinea pigs and also that it could stimulate typical uterine contractions. Seibert[6] pointed out that since the polysaccharide contained 0.3% nitrogen, it had not been conclusively shown that it was the polysaccharide which was responsible for these biological reactions. Enders[35] showed that true anaphylactic shock elicited by the polysaccharide in no way minimized the sensitivity of a tuberculous animal to the skin reaction. This may indicate that the phenomenon of anaphylaxis is not closely connected with that of skin sensitization.

In 1930, Masucci, McAlpine and Glenn[26] isolated a polysaccharide mixture from the culture medium in which *M. tuberculosis* (human strain H37) had been grown. The protein was removed from the fluid by precipitation with neutral lead acetate; lead acetate and ammonia were then added alternately to the filtrate until a precipitate was obtained. The precipitate was dissolved in dilute acid and poured into methyl alcohol, any insoluble matter being removed and discarded. The solution was treated once more with lead acetate and ammonia giving a lead-polysaccharide complex which was decomposed by hydrogen sulfide. A polysaccharide was finally precipitated from solution with absolute alcohol. The product had $[\alpha]_D + 49.8°$ and contained 0.75% nitrogen, 3.3% ash and 32.5% pentose. On hydrolysis, a glucose equivalent of 58.8% (reducing sugar) was obtained. Tests for proteins, glycogen, chloride and sulfate ions were all negative. The polysaccharide was not precipitated from aqueous solution by lead acetate except in the presence of ammonia.

A further quantity of the same substance was prepared by these workers and purified by repeated precipitation with lead acetate and ammonia. The product had $[\alpha]_D + 67.7°$ which changed to $-10°$ after hydrolysis with dilute sulfuric acid for four hours. The amount of reducing sugar present was 75.6%. Mannose, D-arabinose and an unidentified sugar acid were obtained from the hydrolyzate. The substance did not produce a skin reaction, nor did it stimulate antibody formation. It reacted with immune serum in high dilution (1:2,000,000) in the precipitin test. It was claimed that this product was closely related to the polysaccharide isolated by Laidlaw and Dudley.[17]

A similar substance was isolated from the same source by Renfrew,[36] who used substantially the method employed by Masucci, McAlpine and Glenn.[26] The product after purification had $[\alpha]_D + 32°$ and the following

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(35) J. F. Enders, *J. Exptl. Med.*, **50,** 777 (1929).

(36) Alice G. Renfrew, *J. Biol. Chem.*, **89,** 619 (1930).

properties: 1.3% nitrogen; 3% ash; and after hydrolysis, a reducing sugar content of 65%. Two unidentified hydrazine derivatives of sugars and a small quantity of inositol were obtained in the hydrolyzate. The polysaccharide reacted with immune serum in a dilution of 1:1,500,000.

McAlpine and Masucci,[37] by fractional hydrolysis of the product, obtained two sub-fractions. The first had a low dextrorotation, was soluble in alcohol and contained free reducing sugar. It was able to react fairly readily with immune serum. This fraction, it was stated, contained most of the pentose.

The second fraction had a high optical rotation, was insoluble in alcohol and contained a large quantity of D-mannose. It had only a weak precipitation power with immune serum; the biological activity therefore seemed to be associated with the pentose content of the fractions.

These workers[37] also investigated the type-specificity of the culture-medium polysaccharides. It was found that weak cross reactions could occur. The derivative obtained from the metabolism solution of human strain bacilli gave a much stronger reaction with its homologous serum than did the polysaccharide from the bovine strain culture liquor.

These results were confirmed in the main by Dorset and Henley.[38] A polysaccharide was obtained from Long's[39] synthetic medium after growth of *M. tuberculosis*, human strain. This polysaccharide showed a positive biuret test for protein, and underwent hydrolysis only with difficulty. D-Arabinose and D-mannose were identified in the hydrolyzate. No glycuronic acid could be detected. On hydrolysis, the D-arabinose seemed to be liberated before the other constituents. It was concluded from the biological tests that the active principle of tuberculin concerned in eliciting the skin reaction was a protein. This work was repeated with polysaccharides derived from the culture medium of the bovine and avian strains of organisms, and very similar results were obtained. D-Arabinose and D-mannose were also identified in those polysaccharides.

Dienes and Schonheit[40] showed that the filtrates of cultures grown on Long's synthetic medium contained two distinct antigenic fractions, separable by acid precipitation, together with carbohydrate precipitable material. Seibert and Munday[41,42] confirmed the fact that the tuberculin skin reaction was due to proteins present in tuberculin, since fractional hydrolysis of the crude tuberculin precipitate afforded a very

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(37) K. L. McAlpine and P. Masucci, *Am. Rev. Tuberc.*, **24,** 729 (1931).
(38) M. Dorset and R. R. Henley, *J. Am. Vet. Med. Assoc.*, **76,** 696 (1930).
(39) E. R. Long, *Tubercle*, **8,** 111 (1926).
(40) L. Dienes and E. Q. Schonheit, *J. Immunol.*, **18,** 285 (1930).
(41) Florence B. Seibert and B. Munday, *Am. Rev. Tuberc.*, **23,** 23 (1931).
(42) Florence B. Seibert and B. Munday, *J. Biol. Chem.*, **100,** 277 (1933).

potent, water-soluble product, which contained 16% nitrogen and less than 2.7% polysaccharide. Sandor and Schaefer[43] reported that treatment of the culture medium with saturated ammonium sulfate solution precipitated protein-polysaccharide mixtures. Each of these mixtures had equal complement fixing properties. An active haptene-like fraction was obtained from the polysaccharide by fractional precipitation with methyl alcohol. This active constituent amounted to only 2–3% of the original polysaccharide. The polysaccharide, when hydrolyzed, gave a glucose equivalent of 99% reducing sugar. The polysaccharide was a haptene, and it was suggested that it formed part of the biologically reactive lipid or native polysaccharide constituents of the cell. The sensitivity of the haptene in the serological reactions was ascribed to the phenomenal adsorptive capacity of the polysaccharide.

The presence of an acetylpolysaccharide in an aqueous extract of fat-free bacilli (human strain) was reported by Takeda and coworkers.[44] It was claimed that the substance could not only react with immune serum but could elicit antibody formation. It seems probable that this effect may have been due to small traces of protein.

The nature of tuberculin has been extensively investigated by Seibert and her school.[45,6] It has been shown[41] that the polysaccharide associated with tuberculin does not pass through a collodion dialysis membrane. There is reason to believe that the polysaccharide constituent is adsorbed on the protein molecules of tuberculin. Accordingly it has been possible to separate the two by physical means.

Nucleic acid also was found in association with the tuberculin protein. Spiegel-Adolf and Seibert[46] investigated the problem spectrographically, and found that the tuberculin protein ("P.P.D.") precipitated by trichloroacetic acid exhibited an absorption band at 2650–2670 Å. This band was identical with that displayed by deoxyribonucleic acid. Precipitation of the tuberculin protein by ammonium sulfate afforded a product which did not display an absorption band in the ultraviolet region of the spectrum. The substance had a lower phosphorus content than that of products obtained by trichloroacetic acid precipitation. The biological activity was not impaired in any way.

Attempts were made by Gözsy and Vasarhelyi[47] to separate the components of tuberculin by chromatographic analysis using alumina as the adsorbent. No separation occurred, and these authors concluded, there-

(43) G. Sandor and W. Schaefer, *Ann. inst. Pasteur*, **55**, 163 (1935).

(44) Y. Takeda, T. Ota and M. Sota, *Z. Immunitäts.*, **96**, 326 (1939).

(45) Florence B. Seibert, *Bact. Revs.*, **5**, 69 (1941).

(46) M. Spiegel-Adolf and Florence B. Seibert, *J. Biol. Chem.*, **106**, 373 (1934).

(47) B. Gözsy and J. Vasarhelyi, *Z. Immunitäts.*, **97**, 265 (1939).

fore, that their starting material was homogeneous. Another attempt was made by Seibert, Pedersen and Tiselius.[48] Their products still gave rise to fluctuating curves when examined by means of the ultracentrifuge, thus indicating that the fractions were still heterogeneous. Seibert and Watson (in the light of later work[49]) suggested that this heterogeneity was due to interaction between the tuberculin components.

A separation of the nucleic acid, polysaccharide and protein fractions of tuberculin was effected by the Tiselius electrophoretic technique,[48] which was developed for large scale work in this field by Seibert and Watson.[49] The polysaccharide was relatively immobile and thus was easily removed from the protein and nucleic acid. At *p*H 5.0 (or less), the nucleic acid and protein traveled in the electrophoretic tube as a single component. At higher *p*H values, the two tended to move independently.[33] It was probable that some of the protein and nucleic acid was present as nucleoprotein.

Seibert, Pedersen and Tiselius[48] confirmed an earlier observation that the nucleic acid of *M. tuberculosis* was of the deoxyribose type. A modified colorimetric means of determining nucleic acid was devised by Seibert.[33] The nucleic acid has not been found to possess any antigenic properties. Using the electrophoretic methods mentioned, it was found that the polysaccharide component consisted of two fractions. The first was electrophoretically immobile and contained only 0.2% nitrogen. This fraction possessed a higher optical rotation, a lower molecular weight and a more elongated form than the second, which had a low mobility and contained 0.83% nitrogen. Prolonged electrophoresis at *p*H 7.3 did not reduce this figure.

The polysaccharide corresponding to the first, immobile fraction was examined by the Svedberg ultracentrifuge technique.[48] The sedimentation constant, S_{20}, was equal to 1.6×10^{-3} c.g.s. units, and was independent of change of concentration. A 1% solution of the polysaccharide was calculated from data obtained in this investigation to have a polysaccharide content of 0.91%; the polysaccharide thus contained 9% of extraneous matter. The diffusion constant, D_{20}, was found to be 11×10^{-7} and the partial specific volume, 0.619. A molar frictional ratio of 1.5 was obtained. This indicated that the particles were elongated ellipsoids (see p. 320). A molecular weight of 9,000 was calculated as compared with the value 7,300 obtained by Tennet and Watson.[50] The polysaccharide reacted with immune serum in a dilution of 1:6,000,-

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(48) Florence B. Seibert, K. O. Pedersen and A. Tiselius, *J. Exptl. Med.*, **68,** 413 (1938).

(49) Florence B. Seibert and D. W. Watson, *J. Biol. Chem.*, **140,** 55 (1941).

(50) D. M. Tennet and D. W. Watson, *J. Immunol.*, **45,** 179 (1942).

000. It was concluded that the polysaccharides separated electrophoretically were identical with those isolated by chemical means. Moreover, none had a greatly elongated structure, but probably a "closed rigid structure."

IV. POLYSACCHARIDE COMPONENTS OF THE LIPIDS

The unusual features of the lipid constituents of *M. tuberculosis* were first observed by Aronson.[51] The "fat," as the lipids were at first termed, had acid-fast staining properties.[52] Saponification of the "fat" yielded a quantity of soluble matter and a resistant residue. Aronson was able to prepare an acetate from the latter and identified the substance as an alcohol of very high molecular weight. The same author found that organic solvents removed some of the lipids with ease, these consisting largely of free fatty acids.

Further investigation by Kresling[53] confirmed earlier observations that saponification of the "fat" gave an alcohol of high molecular weight. He was unable to detect cholesterol in the tubercle lipids. It was found that ethereal hydrogen chloride would remove the acid-fast staining lipid fraction from the bacillary cell.

Phospholipids were first identified by Hammerschlag[2] and Kresling.[53] Long and Campbell[54] concluded that the waxy substance present in *M. tuberculosis* was a difficultly hydrolyzable ester of an alcohol of high molecular weight. A systematic and thorough examination of the lipids of the bacilli has been undertaken by Anderson and coworkers.[7] He was able to differentiate between four main lipid fractions: (1) phosphatides; (2) acetone-soluble fats; (3) "waxes"; (4) firmly bound lipids.

1. *Phosphatides*[55,56]

This fraction was obtained by precipitation with acetone from an ethereal extract of the cells. It formed acidic colloidal solutions with water and contained 0.37% nitrogen and 2.16% phosphorus. On hydrolysis with 5% sulfuric acid, 33.6% of water-soluble components and 65% of fatty acids were obtained. The hydrolyzate contained glycerophosphoric acid, inositol,[57] D-mannose,[58] and an unidentified hexose, which

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(51) J. D. Aronson, *Berlin. klin. Wochschr.*, **35**, 484 (1898).
(52) E. Klebs, *Zentr. Bakt.*, **20**, 488 (1896).
(53) K. J. Kresling, *Zentr. Bakt.*, **30**, 879 (1901).
(54) E. R. Long and L. K. Campbell, *Am. Rev. Tuberc.*, **6**, 636 (1922).
(55) R. J. Anderson and N. Uyei, *J. Biol. Chem.*, **97**, 617 (1932).
(56) M. C. Pangborn and R. J. Anderson, *J. Biol. Chem.*, **94**, 465 (1931).
(57) R. J. Anderson, *J. Am. Chem. Soc.*, **52**, 1607 (1930).
(58) R. J. Anderson and Alice G. Renfrew, *J. Am. Chem. Soc.*, **52**, 1252 (1930).

formed a phenylosazone identical with that from D-glucose.[59] Hydrolysis of the phosphatide with 1% potassium hydroxide saponified the ester linkages and yielded the carbohydrate constituent, together with salts of fatty acids. The carbohydrate contained D-mannose linked to inositol. It had $[\alpha]_D + 57°$, was reducing to Fehling solution after acid hydrolysis and was named "manninositose." The biological significance of the phosphatide fraction was studied by White.[60] On injection into tuberculous guinea pigs, it was capable of giving rise to acute cellular and general reactions.

2. *Acetone-soluble Fats*

The acetone-soluble fraction was known to contain a large percentage of free fatty acids in addition to the fatty esters. Some of these fatty acids were found by Anderson to be of a type hitherto unknown. In common with the "waxes," this fat did not contain any free glycerides. Alkaline hydrolysis of the fat derived from human strain bacilli yielded a water-soluble carbohydrate. This was identified as trehalose[61] by isolation of the crystalline sugar. Corresponding fats isolated from the bovine and avian[62] strains of bacilli were examined. The presence of glycerol could not be detected nor could the carbohydrate components be identified.

3. *"Waxes"*

The principal lipid constituent of the tubercle bacilli was a wax-like substance. Anderson[63,64] obtained this by chloroform extraction of fat-free bacilli. The preliminary removal of lipids with alcohol and other solvents served to dissolve the free fatty acids, neutral fat and phosphatides. The crude "wax" was fractionated with alcohol or acetone. The purified derivative was a white amorphous powder containing 0.41% nitrogen and 0.77% phosphorus. The substance was very resistant to acid hydrolysis. Continuous treatment with acidified alcohol resulted in very slow hydrolysis. Alkaline hydrolysis proceeded very rapidly, with the formation of ether-soluble components together with a water-soluble polysaccharide.

The polysaccharide readily underwent hydrolysis with dilute acid and contained only traces of nitrogen and phosphorus. The hydrolyzate

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(59) R. J. Anderson, E. G. Roberts and Alice G. Renfrew, *Proc. Soc. Exptl. Biol. Med.*, **27**, 387 (1930).

(60) W. C. White, *Trans. Assoc. Am. Physicians*, **43**, 311 (1928).

(61) M. C. Pangborn and R. J. Anderson, *J. Biol. Chem.*, **101**, 105 (1933); R. J. Anderson and M. S. Newman, *J. Biol. Chem.*, **101**, 499 (1933).

(62) M. L. Burt and R. J. Anderson, *J. Biol. Chem.*, **94**, 451 (1931).

(63) R. J. Anderson, *J. Biol. Chem.*, **83**, 505 (1929).

(64) R. J. Anderson, *J. Biol. Chem.*, **85**, 327 (1929).

had a glucose equivalent of 57% reducing sugar. In the hydrolyzate, 2.1% D-mannose, 17.5% D-galactose, and 35.9% D-arabinose, were identified. Small amounts of D-glucosamine and inositol were present. The non-reducing part of the polysaccharide which reacted with immune serum up to a dilution 1:1,000,000 could not be identified.

The ether-soluble constituents of this purified wax, after saponification, contained fatty acids.[65] The acid-fast staining property was ascribed to "mycolic acid," a long-chain fatty acid. An alcohol, phthiocerol, was also present and was obtained in crystalline form from ethyl acetate. In chloroform it had $[\alpha]_D - 4.8°$. The molecular formula corresponded to $C_{35}H_{72}O_3$ or $C_{36}H_{74}O_3$. The molecule contained two hydroxyl groups and one methoxy group, but the chemical constitution has not yet been settled.

Another wax fraction ("soft wax") obtained by Anderson[64] yielded glycerol, an alcohol, and ether-soluble substances on hydrolysis. The alcohol was again phthiocerol, the presence of which was first reported by Stodola and Anderson.[66]

A third wax subfraction (soluble in both ether and alcohol) was shown[67] to be a mixture of glycerides, carbohydrate esters of fatty acids, and true waxes. Alkaline hydrolysis gave glycerol and the carbohydrate as the water-soluble constituents. The carbohydrate yielded only inositol and D-mannose on hydrolysis with dilute mineral acid.

A similar series of waxes has been isolated from the bovine strain,[68] from the avian strain[69] and from the timothy grass bacillus.[70]

4. *Firmly-Bound Lipids*

Anderson[71] found that a substance of lipid nature was associated with the skeletal material of acid-fast bacilli. This lipid was not removed by neutral organic solvents but could be extracted with an ethereal or chloroform solution of hydrogen chloride. Saponification of these firmly-bound lipids yielded a polysaccharide having properties similar to those of the polysaccharide obtained from the purified wax. Phthiocerol was not detected. The polysaccharide was shown to contain

(65) F. H. Stodola, A. Lesuck and R. J. Anderson, *J. Biol. Chem.*, **126**, 505 (1938).

(66) F. H. Stodola and R. J. Anderson, *J. Biol. Chem.*, **114**, 467 (1936).

(67) C. W. Wieghard and R. J. Anderson, *J. Biol. Chem.*, **126**, 515 (1938).

(68) R. J. Anderson and E. G. Roberts, *J. Biol. Chem.*, **85**, 529 (1930); R. Cason and R. J. Anderson, *ibid.*, **126**, 527 (1938).

(69) R. E. Reeves and R. J. Anderson, *J. Am. Chem. Soc.*, **59**, 858 (1937).

(70) M. C. Pangborn and R. J. Anderson, *J. Am. Chem. Soc.*, **58**, 10 (1936).

(71) R. J. Anderson, M. M. Creighton and R. L. Peck, *J. Biol. Chem.*, **133**, 675 (1940).

D-arabinose, D-mannose and D-galactose. It was able to react with immune serum in high dilution.

TABLE I

Comparison of Properties of Polysaccharides Isolated from Firmly-bound Lipids of Human and Avian Strains of M. Tuberculosis and Leprosy Bacilli

(After Anderson[7])

	Human strain	*Avian strain*	*B. Leprae*
Precipitation titer	1:1,000,000	1:500,000	1:2,000,000
Phosphorus	0.38%	1.04%	Zero %
Nitrogen	0.95	1.81	Trace
Reducing sugar on hydrolysis	57	50.1	50.5
D-Mannose	6.6	27.3	Zero
D-Arabinose	38.7	19.3	41.4
D-Galactose	12.2	3.5	1.0
Inositol	Present	Present	Zero
D-Glucosamine	"	"	"

V. BIOLOGICAL PROPERTIES OF THE POLYSACCHARIDES OF MYCOBACTERIUM TUBERCULOSIS

Three characteristic biological reactions can be ascribed to special polysaccharide components of *M. tuberculosis.* These are the typical skin reaction, usually termed the "*tuberculin reaction,*" certain cellular reactions, and the specific precipitin reaction.

1. *The "Tuberculin Reaction"*

It has been claimed[38,40,41,42] that the causative agent in this reaction is a protein. The polysaccharide associated with this protein does not play any significant role in the reaction. Seibert[41] pointed out that the active principle of protein nature possessed a high degree of stability, as reflected by a retention of biological activity after relatively drastic processes of isolation. It appeared that the biological activity was associated with some small, stable, specific part of the protein complex. Comparison[45] of the various tuberculin preparations showed that the biological function was independent of polysaccharide or nucleic acid constituents. Tuberculin protein readily stimulated antibody formation, as indicated by precipitin reactions,[72] uterine strip contractions[73] and anaphylaxis.[73] Protein fractions which were non-antigenic, but would still cause a local skin reaction, have been isolated.[74] Closer investiga-

(72) Florence B. Seibert, *Am. Rev. Tuberc.,* **21,** 370 (1930).

(73) J. H. Lewis and Florence B. Seibert, *J. Immunol.,* **20,** 201 (1931).

(74) Florence B. Seibert, *J. Infectious Diseases,* **61,** 383 (1932).

tion[48] indicated that the tuberculin protein was not homogeneous and that it consisted of at least two protein components. The component of higher molecular weight possessed a greater antigenic potency than the other. Recent work by Seibert and Nelson[75] has shown that these two protein fractions were electrophoretically slow and fast moving substances, respectively. Both were toxic to tuberculous animals.

2. *Cellular Reactions*

Animal injection of lipid fractions of the tubercle bacillus has been found to cause specific cellular changes in certain organs.[76,77] Sabin, Doan and Forkner[78] demonstrated that giant cell formation could be promoted by injection of a phosphatide fraction. The fatty acids of the tubercle bacillus were able to elicit tubercle formation on injection into animals.

3. *The Specific Precipitin Reaction*

Using the polysaccharide isolated by Mueller[21] from the culture medium of *M. tuberculosis*, Enders[35] embarked on an extensive investigation of the biological properties of this substance. It was claimed that it could induce typical anaphylaxis in guinea pigs. Sabin, Doan and Forkner[78] injected into rabbits polysaccharide fractions of the lipids obtained by Anderson. These did not promote precipitin-antibody formation, induce any specific skin reaction, or cause microscopic or other detectable cellular changes. It was found,[79] however, that they could exert a chemotactic influence (though at the same time deleterious effect) on the leucocytes. These findings were in keeping with those of White.[60] As described earlier, nearly all the polysaccharide fractions of the tubercle bacillus, irrespective of source, have the property of reacting with immune serum in a dilution of the order 1:1,000,000. None of these was able to elicit antibody formation. There would therefore seem to be an immunological similarity between all these polysaccharide fractions, and hence it seems probable that there may be a close structural interrelationship. This view is borne out by the identification of D-arabinose, D-mannose and D-galactose as fundamental sugar units of these polysaccharide fractions.

Evidence has been put forward[37] that the biological activity of these

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(75) Florence B. Seibert and J. W. Nelson, *J. Am. Chem. Soc.*, **65,** 272 (1943).

(76) Florence R. Sabin and C. A. Doan, *J. Exptl. Med.*, **46,** 645 (1927).

(77) T. Wege, *Deut. med. Wochschr.*, **17,** 256 (1891).

(78) Florence R. Sabin, C. A. Doan and C. E. Forkner, *J. Exptl. Med.*, **52,** suppl. 3 (1930).

(79) Florence R. Sabin, F. L. Joyner and K. C. Smithburn, *J. Exptl. Med.*, **64,** 563 (1938).

substances is directly related to their pentose content. The biological properties of the somatic polysaccharide fractions isolated by Heidelberger and Menzel[25] were investigated by Cournand and Lister.[80] They attempted to remove any protein impurities by tryptic digestion. An elementary type of skin reaction given on subcutaneous injection was attributed to the polysaccharide fractions. McCarter and Watson[81] showed that a similar elementary skin reaction was caused by the tuberculin protein, even after rigorous tryptic digestion. These authors also subjected a polysaccharide fraction to acid hydrolysis. The hydrolyzate was still able to promote the elementary type of skin reaction, just as the pure protein did when similarly treated. It seems unlikely, therefore, that the polysaccharide alone was able to stimulate a specific skin reaction, and serological activity appears to be dependent on the presence of combined pentose. McAlpine and Masucci[37] obtained two fractions by mild acid hydrolysis of the polysaccharide. The fraction having the higher pentose content had the higher precipitin titer with immune serum. The polysaccharides of *M. tuberculosis* thus behave as haptenes rather than as antigens.

A preliminary chemical investigation of the polysaccharides of *M. tuberculosis* has recently been undertaken by W. N. Haworth and the authors.[82] It was found that two stable and probably degraded polysaccharide fractions could be isolated from the defatted cells by the action of sodium hydroxide. Both products were serologically active at a dilution of 1:2,000,000; the first fraction ($[\alpha]^{20}_D + 85°$ in water) was derived mainly from the somatic portion of the cell, while the second ($[\alpha]^{20}_D + 27°$ in water) was found in the ether-soluble lipid constituents. Both fractions were intimately associated with deoxyribonucleic acid, which was identified by nitrogen and phosphorus analyses, by the Dische[83] test and by spectrophotometric measurements.

This nucleic acid could be separated only with difficulty from the polysaccharides. Both polysaccharides were smoothly converted into the corresponding neutral methyl ethers, which contained 0.3% nitrogen.

The methylated somatic polysaccharide has been hydrolyzed, and from the mixture of partially methylated sugars so formed, methyl 3,4,6-trimethyl-D-mannopyranoside (25%), methyl 3,4-dimethyl-D-mannopyranoside (20%), methyl 2,3,4-trimethyl-L-rhamnopyranoside (25%) and methyl 3,5-dimethyl-D-arabofuranoside (25%) have been charac-

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(80) A. Cournand and M. Lister, *Proc. Vet. Tuberc. Assoc.*, 156 (1935). *Proc. 3rd. Intern. Congr. Microbiol.*, 621 (1940).

(81) J. R. McCarter and D. W. Watson, *J. Immunol.*, **43**, 85 (1942).

(82) W. N. Haworth, M. Stacey and P. W. Kent, *J. Chem. Soc.*, *in press* (1948).

(83) Z. Dische, *Mikrochemie*, **8**, 4 (1930).

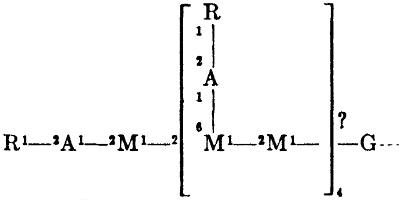
terized. The methylated somatic polysaccharide is nitrogen-containing, and a small quantity of a 2-amino sugar derivative (approximately 5%) has been isolated; the nature of this substance is not yet clear.

The available evidence indicates that in the somatic polysaccharide, L-rhamnose residues are present in the pyranose form and are combined glycosidically as terminal groups. Some of the D-mannose residues are linked through the 1 and 2 positions while other D-mannose residues are linked in the 1, 2 and 6 positions. D-Arabinose is present in the furanose form linked in the 1 and 2 positions.

Having regard to the proportions of these substances present, it is clear that this polysaccharide has a highly branched structure. If there is *one* amino sugar residue present in each polysaccharide molecule, then the other sugar residues are present in the following ratio:

methyl 3,4,6-trimethyl-D-mannoside .	. 5 parts,
methyl 3,4-dimethyl-D-mannoside. . .	. 4 parts,
methyl 3,5-dimethyl-D-arabinoside. . .	. 5 parts,
methyl 2,3,4-trimethyl-L-rhamnoside. . .	. . 5 parts.

Several constitutional formulas are possible in the light of these data. One such formula is depicted.



$$\mathrm{R}^1\text{—}{}^2\mathrm{A}^1\text{—}{}^2\mathrm{M}^1\text{—}{}^2\left[\begin{array}{l} \mathrm{R} \\ {}^1| \\ {}^2| \\ \mathrm{A} \\ {}^1| \\ {}^6| \\ \mathrm{M}^1\text{—}{}^2\mathrm{M}^1\text{—} \end{array}\right]_4 \overset{?}{\text{—}}\mathrm{G}\cdots$$

R = L-Rhamnose
A = D-Arabinose
M = D-Mannose
G = Amino sugar (probable position)

VI. Summary and Discussion

The preceding sections of this review serve to illustrate the complexity of the various polysaccharide fractions isolated from *M. tuberculosis* and its metabolic products.

The methods of isolation, for the majority of these fractions, are based on the solvent action of dilute acids or alkalies. The possibility of the inclusion of extraneous carbohydrate material from the culture fluid has been eliminated by growth of the organism on synthetic media. One school of workers obtained polysaccharide fractions by percolation of fat-free bacilli, over a prolonged period, with dilute acetic acid in the presence of phenol. While this method achieved its purpose by giving a relatively undegraded product, its isolation, and the time taken in the percolation (about six months), may permit profound autolytic changes to occur.

Almost all the polysaccharide fractions (glycogen excepted) isolated from *M. tuberculosis* contained small amounts of nitrogen and phosphorus. Amino sugars have been detected in some polysaccharides, but in the main, the mode of occurrence of the less well-defined constituents remains undetermined. Similarly, some of the polysaccharide fractions have been stated to possess acid properties. Uronic acids have not, however, been identified in the hydrolysis products, nor have any other acidic components been conclusively identified.

It is known that these polysaccharides display "remarkable surface adsorptive capacity" both for organic and inorganic substances. It is therefore possible that these traces of nitrogen and phosphorus are due to strong adsorption between the polysaccharide and small amounts of protein or nucleic acid (or nucleic acid fragments). Such a system is resistant to the usual methods of fractionation, but a separation by electrophoretic means has been claimed.

The principal units from which the polysaccharides are constructed are D-arabinose, D-mannose, D-galactose and L-rhamnose. Inositol is a constituent peculiar to lipid derivatives. The presence of a phosphorylated polysaccharide has been reported.

Comparison of the properties of fractions obtained from the cell and from the metabolism liquid indicates that they are of similar nature. It is not known what part the extracellular enzymes play in modifying the structure of the polysaccharides from the culture medium. The occurrence of D-arabinose in nature is rare, and its presence in the polysaccharides of *M. tuberculosis* indicates the unique metabolic activity of this organism. In other natural materials, arabinose occurs in the L-form (as for example in arabic acid).

The biological role of the isolated polysaccharides appears to be essentially that of haptenes. They are able to react with immune serum in a dilution of the order 1:1,000,000 but cannot elicit antibody formation. In considering these results it should be noted that the fractions still contain traces of nitrogen, and that traces of protein, whether in combination or admixture, may modify biological properties.

The isolated polysaccharides do not, on subcutaneous injection, promote local skin reactions, though they appear to exert a chemotactic effect on the leucocytes. Not all the polysaccharide fractions obtained possessed serological activity. Experiments indicate that the activity is associated with the pentose component of the fractions, and this appears to be true, irrespective of the strain of organism from which the polysaccharides

TABLE II
Summary of Properties of M. Tuberculosis Polysaccharides

Source of polysaccharide	*Bacillary cell*								
	Somatic								
Author	*Laidlaw and Dudley*	*Maxim*	*Heidelberger and Menzel*	*Gough*	*Heidelberger and Menzel*	*Heidelberger and Menzel*	*Chargaff and Schaefer*	*Chargaff and Schaefer*	*Haworth, Stacey and Kent*
Reference	*17*	*18*	*19*	*22*	*25*	*25*	*27*	*27*	*82*
% Water	6.44								
% Ash	9.37	0.05		1.0	1.0	2.0	1.1	47.9	0.6
% Nitrogen	trace	0.5	0.1		0.15	1.65	0.8	0	0.73
% Phosphorus	"			0.8	0.39	0.88	0.9	9.7	0.3
$[\alpha]_D$	+67.8°	+80.2°	+95°	+111°	+79°	+24.2°	+77.4°		+85°
Hydrolysis agent $[\alpha]_D$ (hydrolyzate)		0.5 N H_2SO_4 −24°		2.5% H_2SO_4	−16°	−22°			N H_2SO_4 −22°
Derivatives identified		D-mannose, D-arabinose, sugar acid	D-arabinose, D-mannose	D-arabinose, D-mannose, D-galactose	D-arabinose, D-mannose	D-arabinose (only)	D-arabinose, 8%; D-mannose, 46%; amino sugar, 2.9%	inositol	D-arabinose, 21.9%; D-mannose, 46.8%; L-rhamnose, 24.8%; amino sugar, 5%
% Reducing sugar	51.5	72	87			63.1	77	46.7	
% Pentose	30.6	34.9							
Serological activity	1:6,400,000	1:3,000,000	1:50,000	1:5,000,000	1:2,000,000	1:5,000,000	1:1,000,000	1:2,500,000; 1:1,000,000 after purification	1:2,000,000

TABLE II (*Continued*)

Source of polysaccharide	*Bacillary cell*				*Culture Medium*				
	Lipids								
Author	*Roberts and Anderson*	*Anderson and coworkers*	*Anderson and coworkers*	*Haworth, Stacey and Kent*	*Mueller*	*Renfrew*	*Masucci, McAlpine and Glenn*	*Masucci, McAlpine and Glenn*	*Dorset and Henley*
Reference	*84*	*71*	*71*	*82*	*20*	*36*	*26*	*26*	*38*
% Water							4.50	7.58	
% Ash				0.5	3	3	3.30	2.80	
% Nitrogen	trace	0.95	1.81	1.7	0.3	1.2– 1.35	0.75	0.3	
% Phosphorus	"	0.38	1.04	0.9	trace	trace			
$[\alpha]_D$				+27°	+24.2°	+32°	+49.8°	+67.7°	
Hydrolysis agent $[\alpha]_D$ (hydrolyzate)				N H_2SO_4 −16°	−22.9°			−10°	
Derivatives identified	D-arabinose, 05.9 %; D-mannose, 2.1 %; D-galactose, 17.5 %	D-arabinose, 37.7 %; D-mannose, 6.6 %; D-galactose, 2.2 %	D-arabinose, 19.3 %; D-mannose, 27.3 %; D-galactose, 3.6 %	D-arabinose, D-galactose, D-mannose D-glucosamine		D-arabinose, D-mannose	D-arabinose, D-mannose	D-arabinose, D-mannose, sugar acid	D-arabinose, D-mannose
% Reducing sugar	57				99	65	58.80	75.60	
% Pentose				+	54.8		32.5	29.20	
Serological activity	1:1,000,000	1:1,000,000	1:500,000	1:2,000,000	1:2,000,000	1:1,500,000	1:1,500,000	1:2,000,000	

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(84) E. L. Roberts and R. J. Anderson, *J. Biol. Chem.*, **90**, 33 (1931).

have been isolated. The presence of nucleic acid has not yet been shown to be of serological significance.

The specificity of the polysaccharides isolated from the various strains is of interest. Comparable active fractions prepared from the human, avian and bovine strains show cross reactions; for example, an active avian strain polysaccharide will react with H37 (human strain) antiserum. Type specificity is not a property of the isolated polysaccharide components of the organism.

A detailed structural study should provide invaluable information concerning the nature of the biologically active groupings of these polysaccharides; prior to 1945 no such structural investigations had been carried out. It is probable that the many polysaccharide fractions isolated are constituent parts of one or more main polysaccharide complexes, which have fundamental roles in the immunological behavior of *M. tuberculosis.* An immense field of work is open to carbohydrate chemists.

A comparison of *M. tuberculosis* polysaccharides is given in Table II.

THE CHEMISTRY OF STREPTOMYCIN

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I. Introduction

The antibiotic streptomycin (I), currently considered as second only in importance to penicillin, has been established as a *meso*(3,5- or 2,4-diguanidinotrihydroxycyclohexyl) α-glycoside of a disaccharide, 2-(N-methyl-α-L-glucopyranosaminido)-3-C-formyl-5-desoxy-L-aldopentofuranose. The C-formyl group does not appear to exist in a lactol form and streptomycin is consequently an aldehydo sugar, the designation "aldehydo"[1] denoting an uncyclicized aldehyde function. There remains to be established the configuration of the diguanidino-inositol portion and the complete configuration of the central moiety. There is some indication that the α-glycol group at the 2,3-positions of the pentose portion exists in the *cis*-configuration. This central moiety would there-

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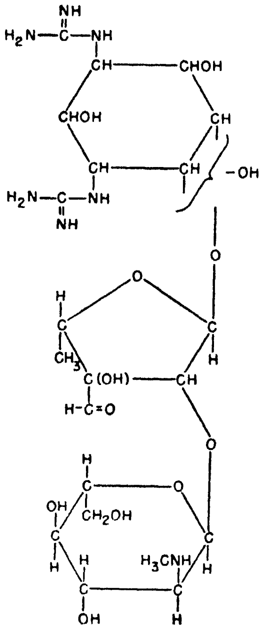
[1] M. L. Wolfrom, *J. Am. Chem. Soc.*, **52**, 2465 (1930).



I

Streptomycin

fore be either 3-C-formyl-5-desoxy-L-ribose or 3-C-formyl-5-desoxy-L-lyxose. The diguanidino-inositol portion has been designated streptidine[2] and the central moiety has been designated both streptose[3] and streptonose.[4] Since the former designation was first offered for publication, it will be adopted in this review.

The progress of science is ever slow. Results are cumulative and never obtained abruptly. They only appear so when viewed by a person remote from the scene. Antibiotic activity was not discovered accidentally by Fleming. A very considerable literature on the subject existed at the time. This literature has been reviewed by Waksman.[5,6] The work on the antagonistic effects of the actinomycetes and substances believed to be elaborated by them is extensive and dates back at least to 1924. What was lacking at that date to bring the work to fruition

(2) N. G. Brink, F. A. Kuehl, Jr., and K. Folkers, *Science*, **102**, 506 (1945).

(3) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, *J. Am. Chem. Soc.*, **68**, 2096 (1946).

(4) J. Fried and O. Wintersteiner, Abstracts of Papers, 110th Meeting, Am. Chem. Soc., September 9–13, p. 15B (1946).

(5) S. A. Waksman and H. B. Woodruff, *J. Bact.*, **40**, 581 (1940).

(6) S. A. Waksman, "Microbial Antagonisms and Antibiotic Substances," The Commonwealth Fund, New York, N. Y. (1945); 2nd edition (1947).

was: the cooperation between the bacteriologist and the chemist; the support of government or industry in the expensive work of growing cultures on a large scale; and the modern techniques of the chemist for handling small quantities of material. Progress in the field of antibiotics can now be expected to proceed at an accelerated pace.

The actinomycetes (order *Actinomycetales*) are nonmotile organisms that may be considered as intermediate forms between bacteria and molds. They occur abundantly in soils, composts, river and lake bottoms, in dust particles, and upon plant surfaces. The order is divided into three families comprising a number of genera. These genera are represented in nature by many thousands of species, of which several hundred have been scientifically characterized. Recent interest in the antibiotics elaborated by the actinomycetes first centered on the substance streptothricin,[7] isolated from *Streptomyces lavendulae* (formerly named *Actinomyces lavendulae*). When this antibiotic was found to exhibit dangerous delayed toxicity effects, attention was turned to streptomycin, which had been isolated from the culture medium of a related organism.

In 1944, Waksman and coworkers[8] obtained a crude concentrate of an antibiotic prepared from cultures of *Streptomyces griseus*[9,10] (formerly named *Actinomyces griseus*), originally isolated from the soil. The substance, which was named streptomycin,[5] proved strongly bacteriostatic against gram-positive and gram-negative organisms and thus showed promise for the treatment of many infections which were resistant to penicillin. Its low toxicity[11,12,13] made it probable that it would develop into a valuable therapeutic agent for a variety of diseases such as tularemia,[14] typhoid fever,[11,15] tuberculosis[16,17] and brucellosis.[18] The his-

(7) S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exptl. Biol. Med.*, **49,** 207 (1942).

(8) A. Schatz, Elizabeth Bugie and S. A. Waksman, *Proc. Soc. Exptl. Biol. Med.*, **55,** 66 (1944).

(9) S. A. Waksman and A. T. Henrici, *J. Bact.*, **46,** 337 (1943).

(10) S. A. Waksman, *Soil Sci.*, **8,** 71 (1919).

(11) H. J. Robinson, Dorothy G. Smith and O. E. Graessle, *Proc. Soc. Exptl. Biol Med.*, **57,** 226 (1944).

(12) Dorothy H. Heilman, *Proc. Soc. Exptl. Biol. Med.*, **60,** 365 (1945).

(13) R. A. Hettig and J. D. Adcock, *Science,* **103,** 355 (1946).

(14) F. R. Heilman, *Proc. Staff Meetings Mayo Clinic,* **19,** 553 (1944).

(15) H. A. Reiman, W. F. Elias and A. H. Price, *J. Am. Med. Assoc.*, **128,** 175 (1945).

(16) W. H. Feldman and H. C. Hinshaw, *Proc. Staff Meetings Mayo Clinic,* **19,** 593 (1944).

(17) A. Schatz and S. A. Waksman, *Proc. Soc. Exptl. Biol. Med.*, **57,** 244 (1944).

(18) D. Jones, H. J. Metzger, A. Schatz and S. A. Waksman, *Science,* **100,** 103 (1944).

tory of the isolation and early development of streptomycin has been reviewed by Waksman and Schatz.[19] A considerable literature exists on the medical aspects of streptomycin.[19a]

The widespread interest in fundamental information regarding the molecular structure of such an important antibiotic facilitated the rapid elaboration of the structure of streptomycin. The substance has been well characterized with regard to its antibacterial activity, protective and toxic effects and other biological properties. The investigations which led to the elucidation of the chemical structure of streptomycin form a noteworthy display of the more modern methods for structural determination and comprise a valuable contribution to carbohydrate chemistry.

II. Production, Isolation and Purification

1. *Production*

a. Streptomyces griseus Growth and Streptomycin Production. Waksman, Schatz and Reilly[20] have reported studies on the metabolism of *Streptomyces griseus* and its production of streptomycin. The growth of the organism was accompanied by a gradual rise in the *p*H and in the ammonia and nitrogen contents of the medium. The growth reached a maximum in stationary cultures in ten days and in aerated, submerged cultures in three to five days. The production and accumulation of streptomycin paralleled the growth of the organism. After maximum activity had been reached, there was a drop in streptomycin activity which was rapid for submerged cultures. The medium became alkaline even in the presence of D-glucose. A weakly alkaline environment[21] is favorable to the formation of streptomycin and acidic conditions are detrimental.

The process of streptomycin formation was greatly facilitated by the addition of meat extract or corn steep liquor to the medium.[8] . Waksman and coworkers[20] pointed out that this addition probably provided an organic substance which served as a precursor of the streptomycin molecule as a whole or of an important group in the molecule, or functioned as a prosthetic group in the mechanism essential for the synthesis of streptomycin. They designated such a substance, required for the

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(19) S. A. Waksman and A. Schatz, *J. Am. Pharm. Assoc.*, **34,** 273 (1945).

(19*a*) See, for instance, the following reviews: R. Murray, T. F. Paine and M. Finland, *New Engl. J. Med.*, **236,** 701 (1947); T. F. Paine, R. Murray and M. Finland, *ibid.*, 748, J. D. Wassersug, *ibid.*, **237,** 22 (1947).

(20) S. A. Waksman, A. Schatz and H. Christine Reilly, *J. Bact.*, **51,** 753 (1946).

(21) S. A. Waksman, Elizabeth Bugie and A. Schatz, *Proc. Staff Meetings Mayo Clinic*, **19,** 537 (1945).

maximum production of an antibiotic agent, the "activity factor" in contradistinction to the accepted term "growth factor," denoting an essential for maximum cell synthesis. The activity factor is not necessary for normal growth of the organism since it will grow equally well on a variety of media with or without meat extract. Since the organism was able to produce slight streptomycin activity in the absence of the meat extract, it was concluded that the activity factor could be gradually synthesized by the organism. When it was provided in the medium in a preformed state, however, as in meat extract or in corn steep liquor, the process of streptomycin synthesis was greatly facilitated. The addition of D-glucose to the culture also aided in increasing the yield of streptomycin.[8]

It became evident that not all strains of *Streptomyces griseus* were capable of producing streptomycin and that streptomycin-producing strains formed active and inactive variants.[22] On the basis that those strains which can actively produce streptomycin would be more resistant to the antibiotic, it was proposed[22] to utilize streptomycin-enriched media for the purification of active cultures of *Streptomyces griseus* from inactive variants.

b. Streptomycin Activity and Bioassay. Chemical methods for the assay of streptomycin have been developed. Since these chemical methods bear a relation to the structure of streptomycin, their discussion will be reserved for a later section of this review. Control analyses in the production of streptomycin are accomplished largely by bioassay techniques. This required the development of methods which would be satisfactory, not only for beers, but also for solutions of streptomycin containing organic solvents and reagents.

Waksman and Reilly[23] have summarized the factors which have a bearing upon the choice of the method to be employed in measuring quantitatively the activity or potency of an antibiotic substance. They have cited literature references to the more commonly used methods. Loo and coworkers[24] have described a suitable method for the quantitative determination of streptomycin by the filter paper disc, agar plate diffusion technique using *Bacillus subtilis* as the test organism. The procedure proved satisfactory in the assay of surface and submerged culture beers and of preparations obtained in isolation and purification

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(22) S. A. Waksman, H. Christine Reilly and D. B. Johnstone, *J. Bact.*, **52**, 393 (1946).

(23) S. A. Waksman and H. Christine Reilly, *Ind. Eng. Chem., Anal. Ed.*, **17**, 556 (1945).

(24) Y. H. Loo, P. S. Skell, H. H. Thornberry, J. Ehrlich, J. M. McGuire, G. M. Savage and J. C. Sylvester, *J. Bact.*, **50**, 701 (1945).

processes. The Food and Drug Administration of the United States Government has defined the unit of streptomycin activity as one microgram of pure free base.[24a] This supplants the various provisional units[19,25] employed before the pure substance became available. Thus the pure free base has an activity of 1000 units per milligram and activities on less pure preparations are expressed as units per milligram or as micrograms per milligram. The stability of streptomycin solutions in relation to the manipulation of working standards has been studied by Oswald and Nielsen,[26] who determined that streptomycin held in solution in concentrations from 100 to 1000 micrograms per milliliter at 10° was stable at *p*H 6.0 to 8.0 for a period of three months. Waksman and coworkers[27] have noted that streptomycin solutions can be largely or completely neutralized or antagonized by various substances such as the common sugars and ascorbic acid.

There exists a rather extensive literature concerned with those specialized bioassay methods developed for the detection and measurement of streptomycin as required in the study of its clinical applications.

There have been several interesting observations regarding the antibiotic activity of streptomycin. Resistance to streptomycin can sometimes be attained by exposure to this agent. Different strains of the same bacterial species may vary greatly in their sensitivity to this antibiotic.[28] Some streptomycin-fast staphylococcal strains showed a change in pigment production and a reduction in the rate of carbohydrate fermentation.[29] Gram-negative bacilli rendered resistant by exposure to streptomycin in broth showed marked pleomorphism and in some instances suffered changes in their biochemical reactions.[30] The development of resistance by *Staphylococcus aureus* to streptomycin did not result in an increase in resistance to streptothricin or penicillin.[31] Each antibiotic has its own characteristic bacterial "spectrum"[5,32] and different substances can be differentiated on the basis of such "spectra."

(24*a*) See *Federal Register*, **12**, 2215, 2217, 2231 (Apr. 4, 1947).

(25) S. A. Waksman, *Science*, **102**, 40 (1945).

(26) E. J. Oswald and J. K. Nielsen, *Science*, **105**, 184 (1947).

(27) W. B. Geiger, S. R. Green and S. A. Waksman, *Proc. Soc. Exptl. Biol. Med.*, **61**, 187 (1946).

(28) S. A. Waksman, H. Christine Reilly and A. Schatz, *Proc. Natl. Acad. Sci. U. S.*, **31**, 157 (1945).

(29) O. E. Graessle and Bettina M. Frost, *Proc. Soc. Exptl. Biol. Med.*, **63**, 171 (1946).

(30) R. Murray, L. Kilham, Clare Wilcox and M. Finland, *Proc. Soc. Exptl. Biol. Med.*, **63**, 470 (1946); see also E. Strauss, *ibid.*, **64**, 97 (1947).

(31) Marguerite Sullivan, G. T. Stahly, J. M. Birkeland and W. G. Myers, *Science*, **104**, 397 (1946).

(32) S. A. Waksman and Elizabeth S. Horning, *Mycologia*, **35**, 47 (1943).

Eisman and coworkers[33,34] have investigated the use of microorganisms made resistant to an antibiotic of known chemical and biological properties in order to differentiate it from an unknown antibiotic of similar bacterial "spectral" analysis. Strains of bacteria which have developed a high resistance to streptomycin have been used to differentiate unknown antibiotics from streptomycin. Some resistant variants have been developed which actually appear to require streptomycin for growth.[34a]

The antibacterial activity of 50 units of streptomycin per milliliter toward *S. aureus* was suppressed by 0.0002 milligram of lipositol per milliliter. The observers[34b] of this interesting phenomenon pointed out that the presence in lipositol of an inositol-galactose structure[34c] suggested the possibility of a metabolite-antimetabolite relationship with streptomycin.

There is presently underway a very considerable amount of bacteriological work having as its objective the elucidation of the mode of action of streptomycin.

2. *Isolation*

Streptomycin was soon recognized as a water-soluble, nitrogeneous, thermostable, acid and alkali labile, levorotatory, basic substance.

The biological inactivation of streptomycin in acid and alkaline solution was first noted by Waksman and coworkers.[8] Regna, Wasselle and Solomons[35] provided detailed information on the effect of *p*H and temperature on commercial and pure streptomycin. Their results indicated that the most favorable conditions for the stability of solutions of streptomycin were at temperatures at or below 28° and between *p*H 3 and 7. Inactivation took place outside of this range. The specific reaction rates of inactivation were determined in both acid and alkaline regions and the reactions were found to be irreversible and first order. Heat greatly increased the rate of decomposition over the entire *p*H scale. Relatively dry neutral salts of streptomycin were stable at 50° over long periods. It appears likely that the inactivation of culture broths after maximum growth is related, at least partially, to the high alkalinity (about *p*H 8.6) which is developed.[20]

(33) P. C. Eisman, R. L. Mayer, K. Aronson and W. S. Marsh, *J. Bact.*, **52**, 501 (1946).

(34) P. C. Eisman, W. S. Marsh and R. L. Mayer, *Science*, **103**, 673 (1946).

(34*a*) C. P. Miller and Marjorie Bohnhoff, *Science*, **105**, 620 (1947); T. Kushnick, C. I. Randles, C. T. Gray and J. M. Birkeland, *ibid.*, **106**, 587 (1947).

(34*b*) I. Rhymer, G. I. Wallace, L. W. Byers and H. E. Carter, *J. Biol. Chem.*, **169**, 457 (1947).

(34*c*) D. W. Woolley, *J. Biol. Chem.*, **147**, 581 (1943).

(35) P. P. Regna, L. A. Wasselle and I. A. Solomons, *J. Biol. Chem.*, **165**, 631 (1946).

All of the published methods for the preliminary concentration of streptomycin from culture broths of *Streptomyces griseus* have employed adsorption from the aqueous solution. Waksman and coworkers[7,8] described the use of activated carbon for the preparation of crude concentrates. The antibiotic fraction was eluted from the charcoal with acidified alcohol (hydrochloric acid in 95% ethanol,[7,8] methanolic hydrogen chloride[36] or methanol-formic acid[37,38]). The eluate was then neutralized and a suitable organic solvent, such as ether, was added to precipitate the streptomycin.[7,8] The use of anhydrous methanolic hydrogen chloride[36] allowed a direct ether precipitation of the active material from the methanol solution. Fried and Wintersteiner[39] precipitated the streptomycin from the acid eluate with phosphotungstic acid. The sensitivity of streptomycin to acid made it imperative that the substance be recovered from the acid eluates as rapidly as possible. LePage and Campbell[40] have used a three step charcoal adsorption process. The research laboratories of the Upjohn Company of Kalamazoo, Michigan, have published the details of their preparation of streptomycin salts.[41] They employ acidified, dilute acetone for elution from the carbon and precipitate the salts by increasing the acetone content. Silcox[42] and Porter[42a] have outlined the method for the commercial production of streptomycin employed by Merck and Co., Inc., of Rahway, New Jersey.

A second antibiotic, designated actidione, has been isolated in crystalline condition from streptomycin beers.[43] Its solubility in organic solvents sharply differentiates it from streptomycin. It is stated to have the formula $C_{27}H_{42}N_2O_7$ and to have little or no activity against bacteria but to be very effective against many yeasts. Waksman and coworkers[20] had previously indicated the probable presence of other antibiotics in such cultures.

3. *Purification*

Crude streptomycin was purified further by precipitation with picric acid and conversion of the picrate to the hydrochloride.[37] Further purification was effected by flowing chromatographic procedures on acid-washed alumina[36,37,44] or on Darco G-60[37] (a commercial activated carbon manufactured by the Darco Corporation, New York, N. Y.).

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[36] H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *J. Biol. Chem.*, **160**, 337 (1945).

[37] F. A. Kuehl, Jr., R. L. Peck, C. E. Hoffhine, Jr., R. P. Graber and K. Folkers, *J. Am. Chem. Soc.*, **68**, 1460 (1946).

[38] F. A. Kuehl, Jr., R. L. Peck, A. Walti and K. Folkers, *Science*, **102**, 34 (1945).

[39] J. Fried and O. Wintersteiner, *Science*, **101**, 613 (1945).

[40] G. A. LePage and Elizabeth Campbell, *J. Biol. Chem.*, **162**, 163 (1946).

[41] M. J. Vander Brook, A. N. Wick, W. H. DeVries, Roberta Harris and G. F. Cartland, *J. Biol. Chem.*, **165**, 463 (1946).

[42] H. Silcox, *Chem. Eng. News*, **24**, 2762 (1946).

[42a] R. W. Porter, *Chem. Eng.*, **53**, No. 10, 94, 142 (1946).

[43] B. E. Leach, J. H. Ford and Alma J. Whiffen, *J. Am. Chem. Soc.*, **69**, 474 (1947).

Fried and Wintersteiner[39] first prepared a crystalline salt of streptomycin. Crude streptomycin phosphotungstate was converted to a crude picrate which was fractionated by flowing chromatography. One or more of the fractions, after removal of the picric acid, yielded a highly active amorphous product from which a crystalline streptomycin reineckate was obtained.

Folkers and coworkers[37,38] found that when sufficiently pure concentrates of streptomycin were treated with methyl orange (the sodium salt of helianthine) a crystalline salt was formed with the sulfonic acid group of this dye. On recrystallization from aqueous methanol, pure streptomycin helianthate was obtained. The helianthate could be converted to streptomycin hydrochloride, sulfate or any other suitable salt for chemical or therapeutic purposes. All of these salts with the common inorganic acids are amorphous. They[37,38] also obtained a crystalline salt of streptomycin and *p*-(2-hydroxy-1-napthylazo)-benzenesulfonic acid from streptomycin hydrochloride and the azo dye Orange II. Streptomycin helianthate was converted directly into a crystalline streptomycin trihydrochloride-calcium chloride double salt.[45] The chemistry of streptomycin was thus put on a crystalline basis.

III. DETERMINATION OF STRUCTURE

The molecular formula of streptomycin was established as $C_{21}H_{37-39}N_7O_{12}$ by elementary analyses of the crystalline streptomycin trihydrochloride-calcium chloride double salt,[45] of crystalline streptomycin trihelianthate and of amorphous streptomycin trihydrochloride of high purity.[37] The composition of crystalline streptomycin reineckate[46] was in agreement with this formula as was also the analysis of the calcium chloride double salt performed in another laboratory.[47]

Streptomycin gave a positive Sakaguchi test[48] and this evidence for the presence of the guanidino group was supported by the fact that alkaline hydrolysis resulted in the formation of ammonia and the disappearance of the test.[36] Two guanidino groups were indicated since only one of the

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(44) G. P. Mueller, Abstracts of Papers, 110th Meeting, Am. Chem. Soc., Chicago, Illinois, September 9–13, p. 14B (1946); *J. Am. Chem. Soc.*, **69**, 195 (1947).

(45) R. L. Peck, N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, A. Walti and K. Folkers, *J. Am. Chem. Soc.*, **67**, 1866 (1945).

(46) J. Fried and O. Wintersteiner, *Science*, **104**, 273 (1946).

(47) I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, *J. Am. Chem. Soc.*, **68**, 2120 (1946); *ibid.*, **69**, 1052 (1947).

(48) S. Sakaguchi, *J. Biochem.* (Japan), **5**, 25 (1925).

three salt groups of streptomycin could be titrated with sodium hydroxide solution.

Potentiometric titrations were carried out on three salts of streptomycin[37] and the data obtained were as follows: helianthate, equivalent weight 1600, calculated (one equivalent of base) for $C_{21}H_{39}N_7O_{12}\cdot 3C_{14}H_{15}N_3O_3S$, 1498; hydrochloride, equivalent weight 740, calculated for $C_{21}H_{39}N_7O_{12}\cdot 3HCl$, 691; hydrobromide, equivalent weight 869, calculated for $C_{21}H_{39}N_7O_{12}\cdot 3HBr$, 825. A cryoscopic molecular weight determination on streptomycin trihydrochloride in water gave a value of approximately 800 for the free base (calculated, 582).[45] Necessary though uncertain corrections for the chloride ion and the non-ideal cryoscopic behavior of the trivalent streptomycin ion were made. Although the high molecular weight of about 700 for streptomycin trihydrochloride rendered difficult the establishment of the exact molecular formula, the combined elementary and molecular weight estimations suggested the structure $C_{21}H_{37-39}N_7O_{12}\cdot 3HX$ for streptomycin salts.[37] Later investigations (see page 358) established the formula $C_{21}H_{39}N_7O_{12}$ for the free base.

1. *Streptidine and Streptobiosamine*

Acid hydrolysis of streptomycin trihydrochloride yielded an optically inactive, strongly basic degradation product which was readily isolated and purified as its picrate.[49,2] This base, designated streptidine,[2] was also obtained as the crystalline sulfate (anhydrous and monohydrate[50] forms), dihydrochloride, dihydriodide, carbonate,[51] and a variety of other crystalline salts.[49] Analytical data on all of these salts indicated the molecular formula $C_8H_{18}N_6O_4\cdot 2HX$. Fried, Boyack and Wintersteiner[51] have described the free base.

Since the molecular formula of streptomycin was shown to be $C_{21}H_{39}N_7O_{12}$ and that of streptidine $C_8H_{18}N_6O_4$, the acid hydrolysis of streptomycin, which would involve reaction with one mole of water, would be expected to yield a basic fraction of molecular formula $C_{13}H_{23}NO_9$. When streptomycin trihydrochloride was treated with methanol containing hydrogen chloride, the biological activity decreased markedly. The mixture of products was separated chromatographically into streptidine dihydrochloride and the amorphous hydrochloride of a base which was named methyl streptobiosaminide hydrochloride dimethyl acetal.[2]

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(49) R. L. Peck, R. P. Graber, A. Walti, Elizabeth W. Peel, C. E. Hoffhine, Jr., and K. Folkers, *J. Am. Chem. Soc.*, **68**, 29 (1946).

(50) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, J. S. Meek, P. S. Skell, W. A. Strong, J. T. Alberi, Q. R. Bartz, S. B. Binkley, H. M. Crooks, Jr., I. R. Hooper and Mildred C. Rebstock, *Science*, **103**, 53 (1946).

(51) J. Fried, G. A. Boyack and O. Wintersteiner, *J. Biol. Chem.*, **162**, 391 (1946).

Analytical and molecular weight data on its crystalline tetraacetyl derivative were in agreement with a composition $C_{13}H_{18}NO_7(CH_3CO)_4(OCH_3)_3$. It was thus evident that the parent compound, streptobiosamine, represented that fraction of streptomycin remaining after the hydrolytic removal of streptidine. The rich oxygen content of streptobiosamine suggested that it was a nitrogen-containing disaccharide-like compound.

2. *The Structure of Streptidine*

Streptidine was best isolated by allowing a solution of streptomycin hydrochloride in *N* sulfuric acid to stand at 37° for forty-five hours. The sulfate monohydrate was precipitated in nicely crystalline form by adding three to five volumes of acetone to the reaction mixture.[50] The substance is also isolable from the alkaline hydrolyzate of streptomycin.[52] Streptidine and its salts have no true melting points and are optically inactive throughout the visible spectrum.[47] The sulfate monohydrate has been definitely characterized by its x-ray diffraction pattern.[47]

The formation of salts containing two univalent acid ions, such as the dihydrochloride and dihydriodide, indicated the presence of two basic groups in streptidine. Potentiometric titration of these basic groups showed them to be of an order of basic strength equivalent to guanidine.[51,53] The presence of guanidino groups in streptidine was suggested by a positive Sakaguchi test[48,50] and was proved by degradation of streptidine to guanidine.[53] Streptidine was oxidized in aqueous solution with potassium permanganate and the liberated guanidine was isolated as the picrate. The yield of the picrate corresponded to 1.3 moles of guanidine per mole of streptidine. Thus, the presence of two guanidino groups in streptidine was established and the nature of all six nitrogen atoms was delineated.

Acetylation of streptidine yielded an acetyl derivative which by analytical and molecular weight data was shown to contain eight acetyl groups and to possess the molecular formula $C_{24}H_{34}N_6O_{12}$. The absence of typical primary amino groups in streptidine (negative Van Slyke reaction) made it doubtful that any of the nitrogen atoms were diacetylated. The ease of hydrolysis of part of the acetyl groups in octaacetylstreptidine suggested that some of them were linked to oxygen. The presence of —OH as well as >N—H groups was supported by infrared absorption. The infrared absorption spectrum of streptidine dihydrochloride was determined on a solid layer deposited from methyl cellosolve (ethylene glycol monomethyl ether) suspension. Bands appeared at

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(52) J. Fried and O. Wintersteiner, *J. Am. Chem. Soc.*, **69**, 79 (1947).

(53) R. L. Peck, C. E. Hoffhine, Jr., Elizabeth W. Peel, R. P. Graber, F. W. Holly, R. Mozingo and K. Folkers, *J. Am. Chem. Soc.*, **68**, 776 (1946).

2.97, 3.38 and 6.10μ. The band at 2.97μ was probably due to —OH or >N—H groups and that at 6.10μ to the >C═N— bond. In the infrared spectral region, a *sym*-tetrachloroethane solution of octaacetylstreptidine showed no band at 2.97μ but exhibited definite strong bands at 5.75 and 6.27μ and a weak band at 6.48μ. The 6.27μ band may have been due to the >C═N— bond, while the 5.75μ band was probably caused by ester carbonyl groups.[49]

Alkaline hydrolysis of streptidine was effected in two stages.[51,53] When streptidine was added to aqueous barium hydroxide solution and the solution was refluxed for about one hour, two moles of ammonia per mole of streptidine were liberated. A crystalline neutral substance was obtained which had the composition $C_8H_{16}N_4O_6$. This neutral urea derivative was named strepturea.[53] When a strongly alkaline solution containing streptidine was refluxed for about twenty-four hours, four moles of ammonia and two moles of carbon dioxide per mole of streptidine were liberated. A new base, designated streptamine,[50,51] with the composition $C_6H_{14}N_2O_4$ was obtained as a crystalline sulfate. Streptamine was characterized as the crystalline free base, the dipicrate, the di-*p*-(*p*-hydroxyphenylazo)-benzenesulfonate, the dihydriodide[53] and the dihydrochloride.[51] All of the nitrogen in streptamine was liberated in the Van Slyke amino nitrogen determination. Streptamine reacted with two equivalents of benzaldehyde to form a crystalline Schiff base. Hexaacetylstreptamine and hexabenzoylstreptamine were obtained as crystalline derivatives and from these compounds the crystalline N,N′-diacetylstreptamine and N,N′-dibenzoylstreptamine were prepared by partial deacylation.[51,53]

Six moles of periodic acid were consumed per mole of streptamine with no formation of formaldehyde.[50,51,53] Under comparable conditions, *meso*-inositol (m. p. 225°) was stated to consume six moles of periodic acid (formic acid was not detected or measured) and to give no formaldehyde while D-mannitol under like conditions consumed five moles of oxidant with the formation of two moles of formaldehyde.[53] It is noteworthy that the periodic acid oxidation of the unsubstituted natural *meso*-inositol (see page 46) had been shown by Fleury, Poirot and Fievet[54] to be anomalous (see page 51).

The consumption of six moles of periodic acid by streptamine without the liberation of formaldehyde showed the absence of primary alcohol groups. This observation along with the fact that analytical data on several salts of streptidine showed it to have two hydrogen atoms less than the number required for a saturated acyclic compound (bromine water, infrared and ultraviolet absorption spectra gave no indication of

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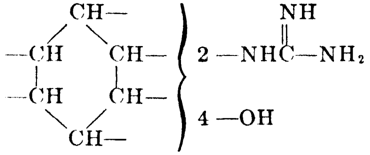
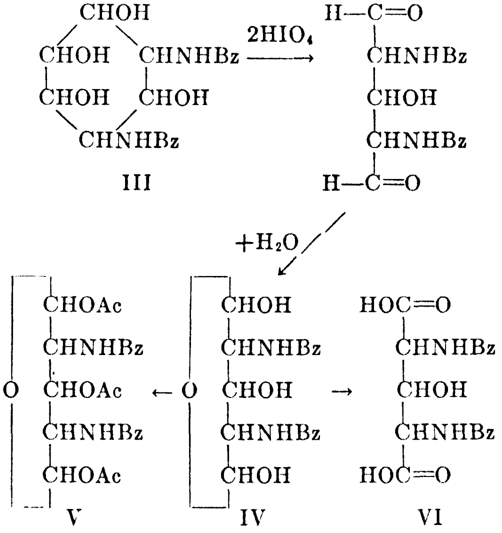
(54) P. Fleury, G. Poirot and Mme. J. Fievet, *Compt. rend.*, **220**, 664 (1945).

unsaturation) suggested that streptidine had a carbocyclic structure (II) in which the two guanidino groups were in the 1,2-, 1,3- or 1,4-positions, with hydroxyl groups substituted at the other four carbon atoms.



II

Periodate oxidation studies on N,N′-diacetylstreptamine, N,N′-dibenzoylstreptamine (III)[50,53] and streptidine (VII)[50] showed that each of these substances consumed only two moles of periodate. A 1,2 arrangement of the two amino or guanidino groups was therefore excluded since this would have required three moles of periodate. This conclusion was substantiated by the failure of streptamine to yield a cyclic ureide when treated with phosgene.[53]



Oxidation of N,N′-dibenzoylstreptamine (III) with periodate yielded a crystalline five carbon dialdehyde which appeared to exist in a hydrated cyclic form (1,3,5-trihydroxy-2,4-dibenzamidotetrahydropyran (IV)), further characterized by its triacetate (V). Oxidation of IV with bromine water yielded a dibenzamidohydroxyglutaric acid (VI).[55] Since these products were to be expected from 1,3-diaminotetrahydroxycyclohexane

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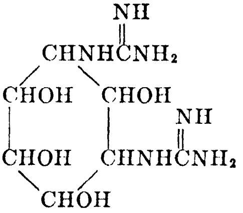
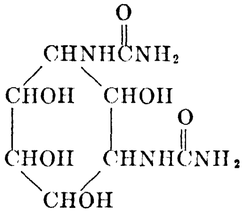
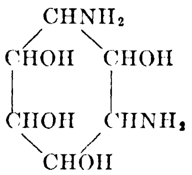
(55) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *Science*, **103**, 540 (1946).

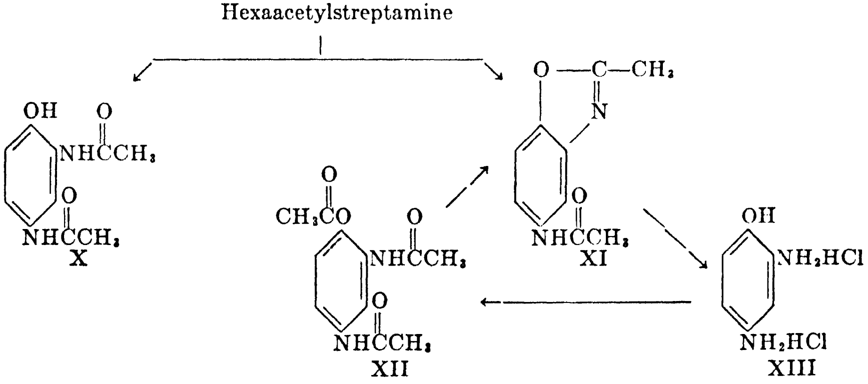
but not from the 1,4-diamino isomer, the data established streptidine as 1,3-diguanidino-2,4,5,6-tetrahydroxycyclohexane (VII). Strepturea and streptamine would then have the structures VIII and IX, respectively.


VII
Streptidine


VIII
Strepturea


IX
Streptamine

The aromatization of streptamine by pyrolysis supported the evidence for the cyclic carbon skeleton, although it is to be noted that structural conclusions drawn from pyrolytic data are ordinarily open to question. Since the aromatization was achieved without elimination of nitrogen, the degradation also yielded information regarding the orientation of the nitrogen atoms. It was found[53] that when hexaacetylstreptamine was heated in a sealed tube at 350° for one hour, 3.5 moles of acetic acid were produced and two crystalline products were obtained. One of the products was shown to be identical with 2,4-diacetamidophenol (X).[56]



Acid hydrolysis of the second product (XI) yielded a dihydrochloride which was identical with an authentic specimen of 2,4-diaminophenol dihydrochloride (XIII). Acetylation of XIII yielded a triacetyl derivative which was identical with an authentic specimen of 2,4-diacetamidophenyl acetate (XII).[56] On heating 2,4-diacetamidophenyl acetate at 350° for one hour a substance was obtained which was identical with the second product of the original pyrolysis and which by its method of syn-

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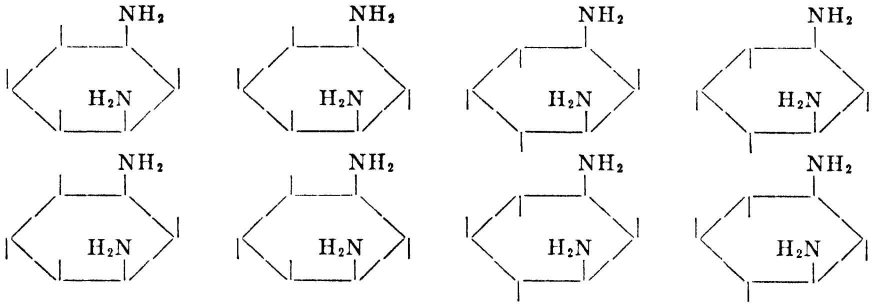
(56) F. Kehrmann and G. Bahatrian, *Ber.*, **31**, 2399 (1898).

thesis was thus identified as 5-acetamido-2-methylbenzoxazole (XI). The combined yield of the crystalline mixture of the diacetyl derivative (X) and the benzoxazole (XI) was about 95% of the theoretical amount.

The following structures represent the eight possible meso forms for streptamine. Since the compound is optically inactive, the two amino groups must lie on the same side of the ring. None of these eight possibilities has been eliminated.



3. *The Structure of Streptobiosamine*

It has already been pointed out (page 346) that the methanolysis of streptomycin yielded, in addition to streptidine, an amorphous, trimethoxy, basic, disaccharide-like substance which was named methyl streptobiosaminide hydrochloride dimethyl acetal. Acetylation of this material yielded a crystalline tetraacetyl derivative whose composition and molecular weight were in agreement with those expected for the second hydrolytic fragment of streptomycin.[2,57]

The inactivation of streptomycin by carbonyl reagents was reported by numerous investigators.[2,27,58] Brink, Kuehl and Folkers[2] showed that when streptomycin was treated with a variety of carbonyl reagents, complete inactivation was observed under *p*H conditions which, in the absence of the reagents, caused only 50% or less inactivation. When streptomycin trihydrochloride was treated with an excess of hydroxylamine hydrochloride in the presence of pyridine, an acidimetric determination of the pyridine hydrochloride[59] formed indicated that streptomycin contained a single free (or potentially free) carbonyl group This conclusion was confirmed by the reaction of streptomycin with hydroxylamine and semicarbazide to form amorphous products with compositions in fair agreement with those expected for monoxime and

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(57) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn and K. Folkers, *J. Am. Chem. Soc.*, **68**, 2557 (1946).

(58) R. Donovick, G. Rake and J. Fried, *J. Biol. Chem.*, **164**, 173 (1946).

(59) W. M. D. Bryant and D. M. Smith, *J. Am. Chem. Soc.*, **57**, 57 (1935).

monosemicarbazone hydrochlorides.[2,57] While streptomycin showed no carbonyl absorption band but exhibited only end absorption in the ultraviolet region, its reaction product with thiosemicarbazide showed the absorption at 2700 Å[58] characteristic of thiosemicarbazones.[60] Since streptidine was known not to contain a carbonyl group,[51] it was concluded that this free carbonyl group, detected in streptomycin, resided in the streptobiosamine portion. It seemed probable that the dimethyl acetal group in methyl streptobiosaminide dimethyl acetal was formed at this carbonyl position. The presence of the third methyl group indicated that in streptomycin the streptidine portion was attached glycosidically to the streptobiosamine moiety.

Streptomycin was inactivated by reducing and oxidizing agents.[61,62] The bacteriostatic activity of this antibiotic for *Escherichia coli* was reduced in the presence of cysteine, sodium thioglycolate, stannous chloride, sodium bisulfite, sodium hydrosulfide, sodium formate and sodium thiosulfate. Cysteine was the most active. Denkelwater, Cook and Tishler[63] found that the cysteine inactivation of streptomycin could be reversed by iodine; presumably cystine was formed during this process. Rake and Donovick[64] inactivated streptomycin with semicarbazide hydrochloride in order to test the sterility of concentrated streptomycin solutions. The inactivation of streptomycin by compounds containing sulfhydryl groups has been discussed by Cavallito.[64a]

The failure of methyl streptobiosaminide hydrochloride dimethyl acetal to yield nitrogen in the Van Slyke assay indicated that the basic nitrogen atom in streptobiosamine was not present as a primary amine. Since this compound formed a N-nitroso derivative and drastic alkaline hydrolysis yielded methylamine, it was concluded that the nitrogen atom in streptobiosamine was present as a methylamino group.[2,57]

*a. N-Methyl-*L*-glucosamine.* Acid hydrolysis of methyl streptobiosaminide dimethyl acetal followed by acetylation yielded a pentaacetyl derivative of a hexosamine. Like all known naturally occurring amino sugars, the nitrogen atom was attached at the 2-position. This was evident when the hexosamine underwent deamination on phenylosazone formation.[65] The phenylosazone melted at the same temperature as L-glucose phenylosazone and the phenylosotriazole prepared from this

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(60) L. K. Evans and A. E. Gillam, *J. Chem. Soc.*, 565 (1943).

(61) A. Bondi, Catherine C. Dietz and E. H. Spaulding, *Science*, **103**, 399 (1946).

(62) R. W. Van Dolah and G. L. Christenson, *Arch. Biochem.*, **12**, 7 (1947).

(63) R. Denkelwater, M. A. Cook and M. Tishler, *Science*, **102**, 12 (1945).

(64) G. Rake and R. Donovick, *Proc. Soc. Exptl. Biol. Med.*, **62**, 31 (1946).

(64*a*) C. J. Cavallito, *J. Biol. Chem.*, **164**, 29 (1946).

(65) F. A. Kuehl, Jr., E. H. Flynn, F. W. Holly, R. Mozingo and K. Folkers, *J. Am. Chem. Soc.*, **68**, 546 (1946); **69**, 3032 (1947).

osazone melted at the same temperature as the corresponding derivative of D-glucose,[66] and the specific rotation was of equal magnitude but opposite in sign.[65] The preparation of L-glucose phenylosotriazole from L-fructose has been reported by Wolfrom and Thompson.[67] Oxidation of the free hexosamine with mercuric oxide[68] gave an acid which had the same melting point reported for N-methyl-D-glucosaminic acid. Again, the rotation was of the same magnitude but opposite in sign. Methylation of D-glucosamine followed by acetylation yielded pentaacetyl-N-methyl-D-glucosamine which had the same melting point, and except for the sign, the same rotation as the pentaacetyl hexosamine derived from streptobiosamine, a derivative later shown[69] to be of the α-L-form. Deacetylation[65] produced the crystalline hydrochloride of N-methyl-α-D-glucosamine. These data[65] and the established configuration of carbon 2 of D-glucosamine[70,71] showed that the hexosamine was N-methyl-2-amino-2-desoxy-L-glucose or N-methyl-L-glucosamine. Later work (see page 360) demonstrated the presence of the pyranose ring in this hexosamine portion of streptomycin.

The characterization of the hexosamine fragment of streptomycin as N-methyl-L-glucosamine was confirmed by synthesis.[65] Hydrolysis of a nitrile resulting from the interaction of L-arabinose, methylamine and hydrogen cyanide yielded an acid which was identical with the acid prepared by oxidation of the natural hexosamine. Thus, the configurations about C3, C4 and C5 of the hexosamine were those at carbons 2, 3 and 4 of L-arabinose (or carbons 3, 4 and 5 of L-glucose). When the synthetic acid was converted to the lactone, reduced and acetylated, the product was found to be identical with the pentaacetyl derivative of the natural hexosamine. Pentaacetyl-N-methyl-α-L-glucosamine was also synthesized by an alternate series of reactions.[65] The L-glucosaminic acid of Fischer and Leuchs[72] was converted into crystalline L-glucosamine hydrochloride by lactonization and reduction. The L-glucosamine was alkylated with dimethyl sulfate and the product was acetylated to give pentaacetyl-N-methyl-L-glucosamine from which crystalline N-methyl-α-L-glucosamine hydrochloride was obtained on acetyl removal.

Wolfrom, Thompson and Hooper[73,74] have found that the reaction

(66) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66,** 735 (1944).
(67) M. L. Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, **68,** 791 (1946).
(68) H. Pringsheim and G. Ruschmann, *Ber.*, **48,** 680 (1915).
(69) M. L. Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, **69,** 1847 (1947).
(70) W. N. Haworth, W. H. G. Lake and S. Peat, *J. Chem. Soc.*, 271 (1939).
(71) E. G. Cox and G. A. Jeffrey, *Nature*, **143,** 894 (1939).
(72) E. Fischer and H. Leuchs, *Ber.*, **35,** 3802 (1902).
(73) M. L. Wolfrom, A. Thompson and I. R. Hooper, *Science*, **104,** 276 (1946).
(74) M. L. Wolfrom, A. Thompson and I. R. Hooper, *J. Am. Chem. Soc.*, **68,** 2343 (1946).

between L-arabinose, methylamine and hydrogen cyanide to produce N-methyl-L-glucosaminic acid, was greatly improved by operating in anhydrous ethanol instead of water. Treatment of a suspension of L-arabinose in absolute ethanol with dry methylamine yielded crystalline N-methyl-L-arabinosylamine. Treatment of this compound, or of a mixture of L-arabinose and methylamine, in absolute ethanol with anhydrous liquid hydrogen cyanide led to the ready crystallization of N-methyl-L-glucosaminic acid nitrile. On hydrolysis of the nitrile with acid followed by base there was obtained, on acidification, N-methyl-L-glucosaminic acid.

It was pointed out[74] that N-methyl-L-glucosaminic acid nitrile was only the second nitrile in the sugar series to be isolated in the cyanohydrin reaction and that extension of the general procedure by which it was prepared should result in the isolation of other members. The nitrile of N-methyl-L-glucosaminic acid showed the same unusual mutarotation as does that of the lower-melting form of D-gluconic acid nitrile.[75] Wolfrom and Thompson[69] have noted that N-methyl-L-glucosaminic acid nitrile hydrochloride was a much more stable compound than the free base.

In the preparation of N-methyl-L-glucosaminic acid nitrile by treatment of an ethanol solution of N-methyl-L-arabinosylamine with hydrogen cyanide, it was to be expected that the epimeric N-methyl-L-mannosaminic acid nitrile would also be formed. Wolfrom and Thompson[69] were able to isolate in crystalline condition the acetylated nitrile of N-methyl-L-mannosaminic acid from this reaction mixture. This compound was converted to crystalline N-methyl-L-mannosaminic acid.

The reduction of N-methyl-L-glucosaminic acid lactone with sodium amalgam in sulfuric acid led to crystalline pentaacetyl-N-methyl-α-L-glucosamine[65,69] from which crystalline N-methyl-L-glucosamine (free base) was prepared.[69] Pentaacetyl-N-methyl-β-L-glucosamine[69] and N-acetyl-N-methyl-L-glucosamine[65] were also isolated.

b. Streptose. Having established the presence of N-methyl-L-glucosamine in the disaccharide-like streptobiosamine, there remained to be determined the structure of the other hexose-like portion.

Streptomycin trihydrochloride was hydrogenated in aqueous solution at atmospheric pressure using platinum as catalyst.[4,52,76,77] About one molar equivalent of hydrogen was absorbed to yield a product which formed a crystalline trihelianthate from which a white powder, designated

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(75) P. E. Papadakis and H. J. Cohen, *J. Am. Chem. Soc.*, **60**, 765 (1938).

(76) R. L. Peck, C. E. Hoffhine, Jr., and K. Folkers, *J. Am. Chem. Soc.*, **68**, 1390 (1946).

(77) Q. R. Bartz, J. Controulis, H. M. Crooks, Jr., and Mildred C. Rebstock, Abstracts of Papers, 110th Meeting Am. Chem. Soc., Chicago, Illinois, September 9–13, p. 8K (1946); *J. Am. Chem. Soc.*, **68**, 2163 (1946).

dihydrostreptomycin trihydrochloride was obtained. Crystalline dihydrostreptomycin reineckate was described[4,52] but the dihydrostreptomycin trihydrochloride did not yield a crystalline calcium chloride double salt.

The antibiotic potency toward numerous organisms and the toxicity of dihydrostreptomycin differed but slightly from those of streptomycin.[52,76–77a] The former was not inactivated by carbonyl reagents under conditions which inactivated streptomycin. This demonstrated that the hydrogenation had reduced the carbonyl group. Dihydrostreptomycin was also not inactivated by cysteine under conditions which caused the inactivation of streptomycin. The retention of biological activity by streptomycin on undergoing such a pronounced change is an observation truly singular in the field of antibiotics. It is apparent that the polarity of the free carbonyl group in streptomycin plays no role in its biological activity. However, if it be remembered that the addition of a wide variety of carbonyl reagents to the carbonyl group can completely destroy the activity of streptomycin, it becomes apparent that these reagents possibly interfere with the antibiotic properties through the additional bulk which they provide in the region of the carbonyl group in the streptomycin molecule.

Hydrogenation of the crystalline streptomycin trihydrochloride-calcium chloride double salt in aqueous solution with platinum oxide catalyst at atmospheric pressure[77] or with Raney nickel catalyst at 100–140 atmospheres and 150°[47] also yielded dihydrostreptomycin. Acid hydrolysis of dihydrostreptomycin gave streptidine and N-methyl-L-glucosamine. Hence, the reduction involved the nitrogen-free moiety, streptose.

Cleavage of streptomycin with ethyl mercaptan and hydrogen chloride yielded streptidine and a new derivative of streptobiosamine, ethyl thiostreptobiosaminide hydrochloride diethyl mercaptal.[3] Treatment of methyl streptobiosaminide hydrochloride dimethyl acetal with ethyl mercaptan and hydrogen chloride readily yielded the same mercaptal hydrochloride. It thus was apparent that the relationship between these two compounds resided simply in the replacement of methoxy by thioethoxy groups on the same carbon skeleton. Acetylation of the mercaptal yielded two anomeric forms of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal.[3,47]

A general method of carbonyl reduction through the stages $>C{=}O \rightarrow >C(SR)_2 \rightarrow >CH_2$ had been reported by Wolfrom and Karabinos,[78] the last step being effected by the general desulfurizing hydrogenolysis

(77a) R. Donovick and G. Rake, *J. Bact.*, **53**, 205 (1947).

(78) M. L. Wolfrom and J. V. Karabinos, *J. Am. Chem. Soc.*, **66**, 909 (1944).

procedure of Bougault, Cattelain and Chabrier.[79] This reductive procedure was then applied[3,47] to both forms of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal and there was obtained one substance which was shown by elementary analysis to have a formula consistent with that required for the replacement of the three thioethoxy groups by hydrogen. Because of the removal of two oxygen atoms and the introduction of two hydrogen atoms into the original disaccharide, the product was designated tetraacetyldidesoxydihydrostreptobiosamine.[47] Hydrolysis of this compound in refluxing concentrated hydrochloric acid yielded N-methyl-L-glucosamine, isolated as the α-L-pentaacetyl derivative.[3,47] Partial deacetylation of tetraacetyldidesoxydihydrostreptobiosamine yielded N-acetyldidesoxydihydrostreptobiosamine, a water-soluble derivative showing no reducing action toward Fehling solution.[3] Didesoxydihydrostreptobiosamine tetraacetate was unchanged on high-pressure hydrogenation at 150° with Raney nickel.[47] Since both of these acetylated forms of didesoxydihydrostreptobiosamine contained the N-methyl-L-glucosamine entity, it was thus established that the hexosamine was joined glycosidically to the streptose portion.

Fried and Wintersteiner[4,52] oxidized streptomycin trihydrochloride with bromine water to an amorphous, antibiotically inactive product The relatively strongly acidic nature of this substance, which they named streptomycinic acid, was demonstrated by electrometric titration. In the infrared spectral region the substance exhibited an absorption maximum at a wave length of 6.06μ, which was partially displaced to 5.81μ when the spectrum was observed in the presence of deuterium oxide and deuterium chloride. A shift of this type is usually found with dipolar ions and was taken therefore as an indication of the expected dipolar ion character of streptomycinic acid.

Degradation of streptomycinic acid with methanolic hydrogen chloride showed that the acid differed from streptomycin merely by the replacement of an aldehyde group by a carboxyl group. This reaction produced streptidine dihydrochloride and the amorphous hydrochloride of a product which contained two methoxy groups, one of which was in the methyl ester of a carboxyl radical and the other was a glycosidic methoxy group. The substance, which was named methyl streptobiosaminidic acid methyl ester hydrochloride,[4,52] consumed one mole of alkali and showed a strong infrared absorption band at 5.77μ, characteristic of an ester carbonyl group. Acetylation of the amorphous acid yielded a crystalline tetraacetyl derivative, the composition of which was

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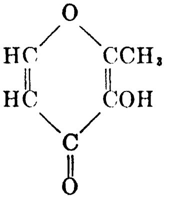
(79) J. Bougault, E. Cattelain and P. Chabrier, *Bull. soc. chim.*, [5] **5**, 1699 (1938); *ibid.*, [5] **7**, 780, 781 (1940).

in good agreement with that expected for the corresponding derivative of streptobiosamine. Ammonolysis of the methyl ester, followed by acetylation, yielded an amorphous methyl tetraacetylstreptobiosaminidic acid amide. It therefore became evident that the free carbonyl group in the streptose portion of streptomycin was aldehydic in nature.

Degradation of dihydrostreptomycin in methanolic hydrogen chloride yielded methyl dihydrostreptobiosaminide, which formed a pentaacetyl derivative on acetylation.[4,47,52,57,77] Both anomers of this compound were isolated.[57] The crystalline methyl pentaacetyldihydrostreptobiosaminide was also obtained by selective hydrolysis of the non-glycosidic acetal group in methyl streptobiosaminide dimethyl acetal followed by catalytic hydrogenation and acetylation.[52]

Treatment of streptomycin with *N* sodium hydroxide for three minutes at 100° or for eighteen hours at 40° yielded a weakly acidic substance which was identified as maltol (XIV).[80] Streptidine is simultaneously liberated.[52] The presence of a terminal methyl group in the parent substance, streptomycin, was established by a CH_3—C group assay.[47,57] It was evident that this methyl group was contained in the streptose portion and that maltol was formed through the alkaline degradation of this central moiety.



XIV
Maltol

Wolfrom and coworkers[47] had found that the mercaptolysis of the crystalline calcium chloride double salt of streptomycin trihydrochloride yielded, after chromatographic purification,[81] two anomeric forms of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal. Mercaptolysis of dihydrostreptomycin yielded an amorphous ethyl dihydrothiostreptobiosaminide hydrochloride which formed crystalline N-acetyl[3] and pentaacetyl[82] derivatives. The ability of the ring in this latter compound to resist hydrolysis and to form a thioglycoside instead of a thio-

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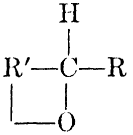
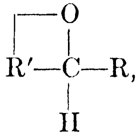
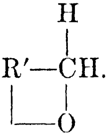
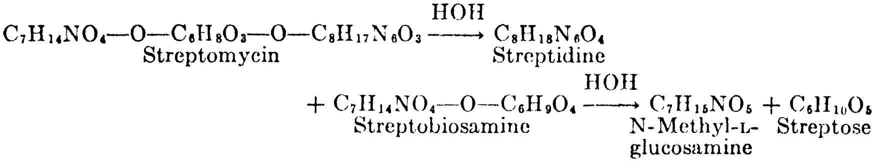
(80) J. R. Schenck and M. A. Spielman, *J. Am. Chem. Soc.*, **67**, 2276 (1945).

(81) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *J. Am. Chem. Soc.*, **67**, 527 (1945).

(82) R. U. Lemieux, W. J. Polglase, C. W. DeWalt and M. L. Wolfrom, *J. Am. Chem. Soc.*, **68**, 2748 (1946).

acetal suggested that it contained the same ring structure which resisted cleavage on the mercaptolysis of streptomycin. Hydrogenolysis of ethyl pentaacetyldihydrothiostreptobiosaminide yielded pentaacetyldesoxydihydrostreptobiosamine.[82] This same compound was obtained on reducing, through the ethyl thioglycoside, the two anomeric forms of methyl pentaacetyldihydrostreptobiosaminide.[57] Hydrogenolysis of either anomeric form of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal with Raney nickel catalyst, followed by reacetylation, yielded in each case an identical tetraacetyldidesoxydihydrostreptobiosamine.[47,82] These anomers are therefore concerned with the asymmetry of the glycosidic carbon attaching the streptobiosamine entity to streptidine. Since the anomeric thioglycosides of a ketose would be expected to yield, on hydrogenolysis, different desoxy derivatives,  $R'\!-\!\overset{H}{\underset{O}{C}}\!-\!R$ and  $R'\!-\!\overset{O}{\underset{H}{C}}\!-\!R$, it became apparent that the thioglycoside groups, in the two anomeric ethyl tetraacetylthiostreptobiosaminide diethyl mercaptals, were formed at an aldehydic position. For aldoses, both anomeric forms of the thioglycoside would yield  $R'\!-\!\overset{H}{\underset{O}{C}}H$. The presence of two terminal methyl groups in didesoxydihydrostreptobiosamine[82] showed that the diethyl mercaptal group, in ethyl tetraacetylstreptobiosamine diethyl mercaptal, also occupied an aldehydic position. It was thus evident that streptose contained two aldehyde groups and a terminal methyl group. Further proof for this conclusion was provided by quantitative measurements of hypoiodite oxidation.[82] Although this reagent caused some general oxidation, time curves showed definite breaks at the following consumptions expressed in atoms of oxygen: streptomycin, 1; streptomycin hydrolyzate, 2; dihydrostreptomycin, 0; dihydrostreptomycin hydrolyzate, 1. The hydrolyzates were prepared under conditions which cleaved only the glycosidic bond linking streptidine to the streptobiosamine residue.[52]

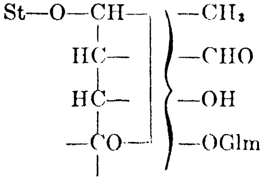
Elementary analyses on a variety of streptobiosamine derivatives coupled with the known structure for streptidine definitely established the formula $C_{21}H_{39}N_7O_{12}$ for streptomycin.[3,47] Since streptidine was established as $C_8H_{18}N_6O_4$ and N-methyl-L-glucosamine was $C_7H_{15}NO_5$, then free streptose had the molecular formula $C_6H_{10}O_5$.



$$\underset{\text{Streptomycin}}{C_7H_{14}NO_4\text{—O—}C_6H_8O_3\text{—O—}C_8H_{17}N_6O_3} \xrightarrow{HOH} \underset{\text{Streptidine}}{C_8H_{18}N_6O_4}$$

$$+ \underset{\text{Streptobiosamine}}{C_7H_{14}NO_4\text{—O—}C_6H_9O_4} \xrightarrow{HOH} \underset{\text{N-Methyl-L-glucosamine}}{C_7H_{15}NO_5} + \underset{\text{Streptose}}{C_6H_{10}O_5}$$

Since streptose contained three terminal groups (two aldehyde and one terminal methyl group), it was evident that, if streptose were in fact a hexose, it must possess a branched chain structure bearing three hydroxyl groups. Then the partial structure XV could be written for streptomycin.



XV

St = Streptidine, Glm = N-methyl-L-glucosamine

The detection of a free hydroxyl group in the streptose portion of streptomycin would have established the carbon skeleton of streptose as a branched chain. This however proved a difficult matter. The preparation of the first eight different crystalline acetyl derivatives of streptobiosamine degradation products, did not yield chemical evidence for a free hydroxyl group. The acetyl groups in these compounds, see Table IV (page 383) can all be attributed to the four esterifiable positions in the N-methyl-L-glucosamine portion and, for the dihydrostreptobiosamine derivatives, to the additional primary hydroxyl group formed by the reduction of the aldehyde group to the carbinol stage.

However, solutions of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal[3] and methyl tetraacetylstreptobiosaminide dimethyl acetal[57] in high concentrations (about 50%) in *sym*-tetrachloroethane showed infrared absorption bands at 2.85μ and 2.75μ, the —OH and $>$N—H region. Differential acetyl estimations[83] had shown that in the latter compound three of the acetyl groups were attached to oxygen and the fourth to nitrogen. Consequently, the nitrogen atom in each derivative was present as $>$CHN(CH$_3$)COCH$_3$ and there could be no $>$N—H

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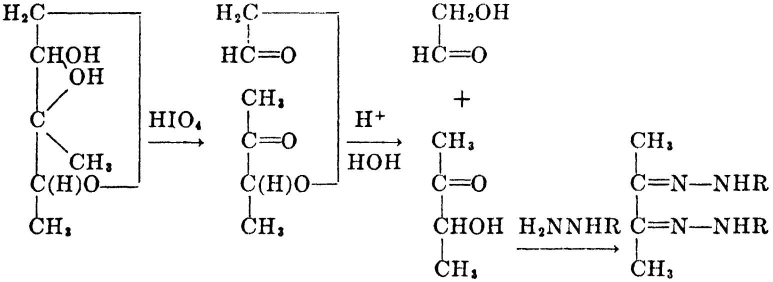
(83) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *J. Am. Chem. Soc.*, **58**, 490 (1936).

group. The infrared absorption data therefore indicated the presence of a free hydroxyl group which resisted acetylation. This conclusion was confirmed by the Zerewitinoff active hydrogen determination.[57] Methyl tetraacetylstreptobiosaminide dimethyl acetal liberated one mole of methane. Infrared absorption spectra and Zerewitinoff analyses of the anomeric forms of methyl pentaacetyldihydrostreptobiosaminide indicated the presence of a free, unacetylable hydroxyl group in both of these compounds.[57]

Acid hydrolysis of tetraacetyldidesoxydihydrostreptobiosamine[3,47] yielded N-methyl-L-glucosamine and crystalline didesoxydihydrostreptose.[84] The latter substance yielded a di-*p*-nitrobenzoate. This compound was the first derivative in which the difficultly esterifiable hydroxyl group in the streptose portion of streptomycin was acylated. A branched carbon skeleton was established therefore for streptose and streptose was shown to be a hexose containing two aldehyde groups.

Didesoxydihydrostreptose was oxidized with one mole of periodic acid and the product was hydrolyzed with acid. Treatment of the solution with excess amounts of substituted hydrazines gave osazones of diacetyl.[84] These data showed that the two terminal methyl groups[82] in didesoxydihydrostreptose were part of a four carbon fragment which was resistant to cleavage by periodic acid. Since the compound must have contained a ring structure with the oxygen atom bridging from the first carbon atom and since the compound contained an α-glycol group, it must have been 3,4-dihydroxy-2,3-dimethyl-tetrahydrofuran (XVI). Periodic acid oxidation[84] of XVI therefore yielded the compound XVII which on hydrolysis liberated acetoin (XVIII) which gave osazones of diacetyl (XIX).



XVI XVII XVIII XIX

Periodate oxidations of N-acetyldidesoxydihydrostreptobiosamine indicated a pyranose ring structure for the N-methyl-L-glucosamine por-

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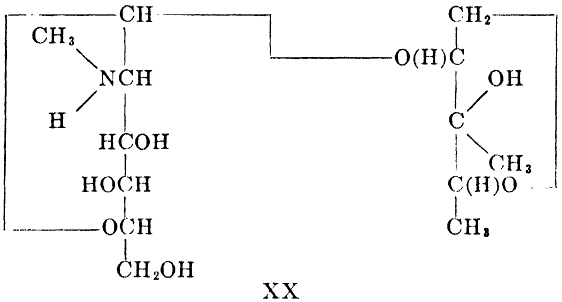
(84) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn and K. Folkers, *J. Am. Chem. Soc.*, **68**, 2405 (1946).

tion.[84] The primary rapid reaction appeared to be with one mole of periodate, and neither formic acid nor formaldehyde could be isolated.

The high resistance toward acetylation of the free hydroxyl group in tetraacetyldidesoxydihydrostreptobiosamine suggested that it was tertiary in nature. On this basis, N-methyl-L-glucosamine would be attached to the C2 position of streptose[84] and didesoxydihydrostreptobiosamine would have the structure XX.



XX

Hydrolysis with aqueous mercuric chloride in the presence of cadmium[85] carbonate removed the thioethoxy groups in ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal. Crystalline tetraacetylstreptobiosamine was obtained in this manner.[86] Bromine oxidation of tetraacetylstreptobiosamine in the presence of strontium carbonate yielded an acid which, after reacetylation, was obtained as the crystalline pentaacetyl derivative. The analytical data and the results of potentiometric titrations for this oxidation product, designated pentaacetylstreptobiosamic acid monolactone,[86] were in agreement with the formula, $C_{13}H_{16}NO_{10}(CH_3CO)_5$, required for a dibasic acid monolactone. A crystalline monomethyl ester of the substance was prepared. The established formula shows that all hydroxyl groups present are acetylated or lactonized.

Hydrolysis of pentaacetylstreptobiosamic acid monolactone in refluxing 2.5 *N* hydrochloric acid yielded a crystalline compound derived from the streptose moiety. This compound was designated streptosonic acid monolactone and was further characterized as its diacetate.[86] The hydrolyzate was shown to contain N-methyl-L-glucosamine.

Analysis of streptosonic acid monolactone showed a composition corresponding to the formula $C_6H_8O_6$.[86] The formulation of this compound as a monolactone of a dibasic acid was based on its behavior during potentiometric titration, when neutral equivalents of 175 and 87.5 were

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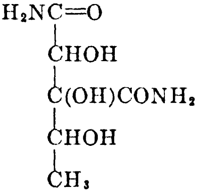
(85) M. L. Wolfrom, *J. Am. Chem. Soc.*, **51**, 2188 (1929).

(86) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, *J. Am. Chem. Soc.*, **68**, 2679 (1946).

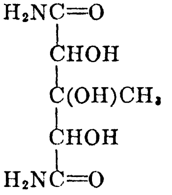
observed (theoretical values were 176 and 88) and upon infrared analysis, the compound showing absorption at 5.80μ (carboxyl group) and at 5.62μ (lactone). Further, a diamide having the formula $C_6H_{12}N_2O_5$ was prepared from the lactone, confirming the dibasic character of streptosonic acid. Chromic acid oxidation of streptosonic acid monolactone gave one mole of acetic acid and the presence of the expected terminal methyl group was further evidenced by a positive iodoform reaction.

On the basis of the structure of didesoxydihydrostreptose (XVI) it was evident that the structure of streptosonic acid diamide was either XXI or XXII. The presence of three adjacent hydroxyl groups was confirmed by periodate oxidation of both streptosonic acid monolactone and streptosonic acid diamide. Two moles of oxidizing agent were consumed in each case.[86]



$$\begin{array}{l} H_2NC{=}O \\ \quad | \\ \quad CHOH \\ \quad | \\ \quad C(OH)CONH_2 \\ \quad | \\ \quad CHOH \\ \quad | \\ \quad CH_3 \end{array}$$

XXI

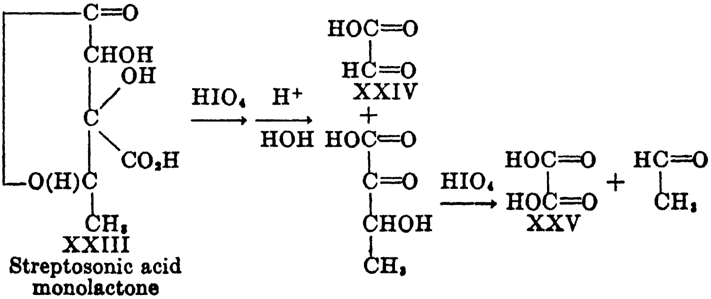


$$\begin{array}{l} H_2NC{=}O \\ \quad | \\ \quad CHOH \\ \quad | \\ \quad C(OH)CH_3 \\ \quad | \\ \quad CHOH \\ \quad | \\ H_2NC{=}O \end{array}$$

XXII

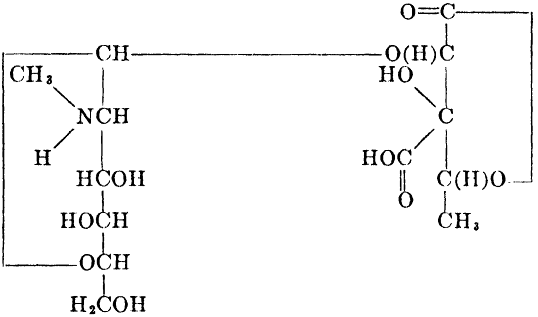
Investigation[86] of the products obtained by the oxidation of the diamide with two moles of sodium metaperiodate showed that no volatile acid was produced. This would exclude structure XXII since it would be expected to yield acetic acid. The presence of a volatile aldehyde, identified as acetaldehyde, was in accord with structure XXI.

Confirmatory evidence for streptosonic acid diamide existing as structure XXI was obtained by a study of the oxidation products of streptosonic acid monolactone (XXIII).[86] When this compound was oxidized with two moles of periodic acid, two of the cleavage products were identified as glyoxylic acid (XXIV) and oxalic acid (XXV). The suggested course of the oxidation, on the basis of structure XXIII, is shown



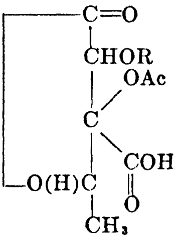
below. There was no evidence for the position of the lactone ring but the γ-position was preferred on the basis of the structure of the known sugar lactones.

Assuming structure XXIII for streptosonic acid monolactone and structure XX for didesoxydihydrostreptobiosamine, streptobiosamic acid monolactone would be XXVI.

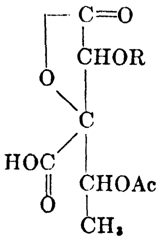


XXVI

Unlike the other streptobiosamine derivatives (Table IV, page 383) streptobiosamic acid monolactone formed a pentaacetyl derivative[86] rather than a tetraacetyl compound. Also, acetylation of streptosonic acid monolactone yielded a diacetyl derivative.[86] It was pointed out[86] that either the ordinarily resistant tertiary hydroxyl group had been acetylated in these compounds (XXVII) or that the lactone ring had shifted from the γ- to the β-position, during the acetylation, with the introduction of an acetyl group at carbon atom four (XXVIII). Whatever the positions of the ester groupings in pentaacetylstreptobiosamic acid monolactone, this compound comprised the only derivative of streptobiosamine in which the tertiary hydroxyl group had undergone esterification.



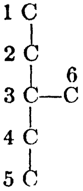
XXVII



XXVIII

R = Acetyl or N-methyl-L-glucosamine

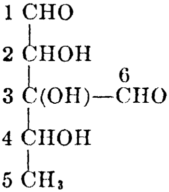
Since the structure of didesoxydihydrostreptose (XVI) required the free aldehyde group in the streptose portion of streptomycin to involve either carbon 5 or 6 of the carbon skeleton XXIX and since the positions of the carboxyl groups in streptosonic acid monolactone (XXIII) limited the positions of the aldehyde groups to carbon atoms 1 and 6 of formula



$$\begin{array}{lll} 1 & \mathrm{C} & \\ & | & \\ 2 & \mathrm{C} & \\ & | & \ \ 6 \\ 3 & \mathrm{C} & \text{—C} \\ & | & \\ 4 & \mathrm{C} & \\ & | & \\ 5 & \mathrm{C} & \end{array}$$

XXIX

XXIX. it followed that the acyclic form of streptose would be XXX.[86]



$$\begin{array}{lll} 1 & \mathrm{CHO} & \\ & | & \\ 2 & \mathrm{CHOH} & \\ & | & \qquad 6 \\ 3 & \mathrm{C(OH)} & \text{—CHO} \\ & | & \\ 4 & \mathrm{CHOH} & \\ & | & \\ 5 & \mathrm{CH_3} & \end{array}$$

XXX

The structure of didesoxydihydrostreptose has provisionally established the structure of didesoxydihydrostreptobiosamine as XX on the assumption that the difficultly esterifiable free hydroxyl group in the streptose portion of the streptobiosamine derivatives was a tertiary hydroxyl group. All of the accumulated evidence indicated that in streptomycin one of the aldehyde groups in XXX was free and that the other was lactolized and attached to streptidine; and that the hexosamine was united glycosidically to one of the hydroxyl groups, probably at the C2 position of the streptose moiety. However, more definite evidence for the point of attachment of the hexosamine was desirable. An example wherein a polyhydroxy compound, containing a tertiary hydroxyl group, resists acetylation is to be found in the formation of a tetraacetyl, as well as a hexaacetyl, derivative on acetylation of an inactive di-C-methylinositol.[86a] On the other hand, a branched chain sugar "isosaccharinose," obtained by the sodium amalgam reduction of an isosaccharinic acid lactone,[86b] contained a tertiary hydroxyl group which was found to be acetylable.[86c]

Now the hydrogenation of streptomycin to dihydrostreptomycin involved the reduction of the free carbonyl group to carbinol. Therefore, the allocation of the primary alcohol group so formed to the 1 or 6 posi-

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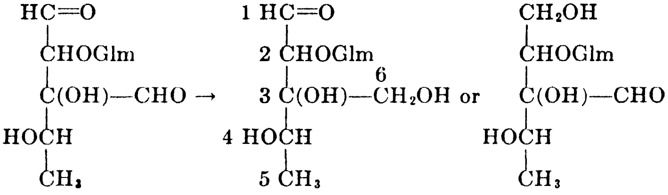
(86a) A. W. K. De Jong, *Rec. trav. chim.*, **27**, 257 (1908).

(86b) P. Schorigin (Shoruigin) and N. N. Makarowa-Semljanskaja (Makarov-Zemlyanskii), *Ber.*, **66**, 387 (1933).

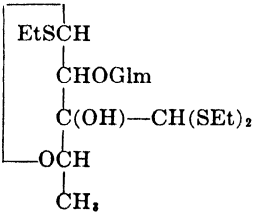
(86c) R. U. Lemieux and M. L. Wolfrom, *J. Am. Chem. Soc.*, *in press* (1948).

tion of dihydrostreptobiosamine, XXXII or XXXI, would establish the point of attachment of streptidine.

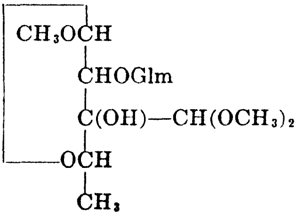


XXXI XXXII

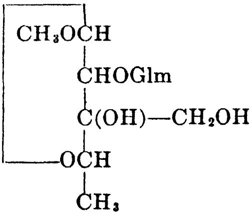
The two possible structures for dihydrostreptobiosamine (XXXI and XXXII) with the N-methyl-L-glucosamine situated at C2, could yield on methanolysis a variety of different glycosidic structures. However, several of these possible structures for methyl dihydrostreptobiosaminide were excluded since Fried and Wintersteiner[52] were able to catalytically hydrogenate a methyl streptobiosaminide hydrochloride, obtained by the selective hydrolysis of the dimethyl acetal group in methyl streptobiosaminide dimethyl acetal, to methyl dihydrostreptobiosaminide. That is, the ring structure in the latter compound does not involve the primary hydroxyl group and the dimethyl acetal group in methyl streptobiosaminide dimethyl acetal occupied that aldehyde position which is free in streptomycin. Furthermore, the structure of didesoxydihydrostreptobiosamine (XX) together with the structure of streptose (XXX) established the structure of ethyl thiostreptobiosaminide diethyl mercaptal as XXXIII and by analogy, methyl streptobiosaminide dimethyl acetal would be expected to have the structure XXXIV. The ring structure and the position of the primary hydroxyl group in methyl dihydrostreptobiosaminide would therefore be as represented in XXXV. This conclusion was confirmed and the point of attachment of the hexosamine was established at the C2 position of the streptose portion by periodate oxidation studies.



XXXIII



XXXIV



XXXV

Methyl N-acetyldihydrostreptobiosaminide and methyl N-acetylstreptobiosaminide dimethyl acetal were subjected to periodate oxidation.[87]

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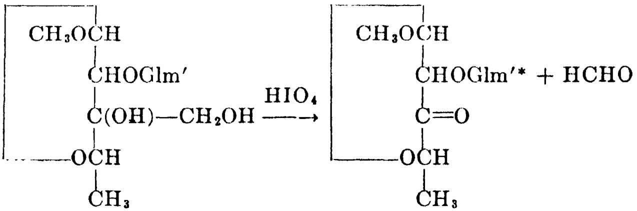
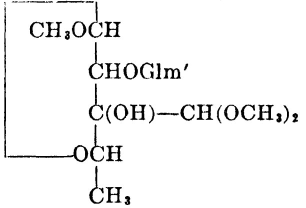
(87) R. U. Lemieux, C. W. DeWalt and M. L. Wolfrom, *J. Am. Chem. Soc.*, **69**, 1838 (1947); *ibid., in press* (1948).

The oxidation of the former differed from that of the latter in the rapid consumption of one mole of periodate and the concomitant formation of one mole of formaldehyde. This oxidation necessarily had its origin in the streptose portion of the methyl dihydrostreptobiosaminide and indicated the presence, in this moiety, of an α-glycol group one hydroxyl of which was primary. This result therefore offered final proof that the hexosamine was attached to the C2 position of the streptose portion and methyl N-acetyldihydrostreptobiosaminide would have the structure XXXVI. The oxidation may be formulated as follows.



XXXVI XXXVII

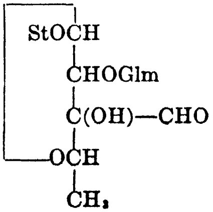
* The N-acetyl-N-methyl-L-glucopyranosamine portion (Glm′) was oxidized slowly.



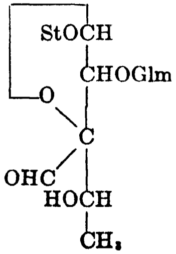
XXXVIII

Methyl N-acetylstreptobiosaminide dimethyl acetal (XXXVIII) did not contain an acyclic α-glycol group and consequently did not show the initial rapid consumption of one mole of periodate.

There thus remain only two possible structures for streptomycin, XXXIX and XL. Either of these possible structures could account for all data so far accumulated, since these data rest upon hydrolytic (methanolysis or mercaptolysis) operations which could involve a change in the



XXXIX

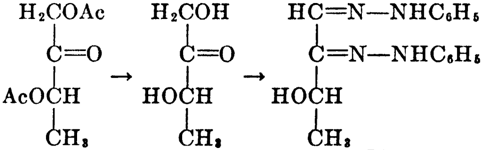


XL

lactol ring of the streptose portion (see page 381). A decision between the structures XXXIX and XL was readily provided by periodate oxidation.[87] When dihydrostreptomycin was oxidized with 1.5 moles of periodate, 0.5 mole of formaldehyde was formed. A similar oxidation of streptomycin yielded no formaldehyde. These results therefore demonstrated that dihydrostreptomycin possessed the 1,4-furanose ring as established for methyl dihydro-L-streptobiosaminide (XXXV) and streptomycin was thus XXXIX, a 2-(N-methyl-L-glucopyranosaminido)-strepto-1,4-furanoside of a meso streptidine.

Fried, Walz and Wintersteiner[88] isolated 4-desoxy-L-glycero-tetrose[90] phenylosazone (XLII) from the reaction of streptobiosamine hydrochloride with phenylhydrazine and thus demonstrated that streptose was configurationally an L sugar. The levorotation ($[\alpha]^{25}D$ −37°, water[86]) of streptosonic acid monolactone (XXIII) might also be adduced as contributory evidence for the L configuration of the penultimate carbon of streptose, should Hudson's lactone rule[89] be applicable to this rather complex structure.

The structure of the osazone was established by synthesis.[88] 1,3-Diacetyl-4-desoxy-L-glycerotetrulose[90] (XLI) was synthesized from acetyl-L-lactyl chloride prepared from L-(*dextro*)-lactic acid, through 3-acetyl-1-diazo-1,4-didesoxy-L-glycerotetrulose following the general procedure of Wolfrom, Waisbrot and Brown[91] for the synthesis of ketoses. When this diacetyl derivative was hydrolyzed with dilute ammonia and the hydrolyzate was treated with acetic acid and phenylhydrazine, a phenylosazone (XLII) was isolated which was identical with that obtained from streptobiosamine. Its optical rotation was determined in pyridine solution. The preparation of the phenylosazone from streptobiosamine was well reproducible, with yields of 25–30% of the theoretical in terms of chromatographed material.



$$\begin{array}{c} H_2COAc \\ | \\ C{=}O \\ | \\ AcOCH \\ | \\ CH_3 \end{array} \rightarrow \begin{array}{c} H_2COH \\ | \\ C{=}O \\ | \\ HOCH \\ | \\ CH_3 \end{array} \rightarrow \begin{array}{c} HC{=}N{-}NHC_6H_5 \\ | \\ C{=}N{-}NHC_6H_5 \\ | \\ HOCH \\ | \\ CH_3 \end{array}$$

XLI XLII

Indication that the formation of the phenylosazone (XLII) was connected with the free aldehyde group in streptomycin was provided by the fact

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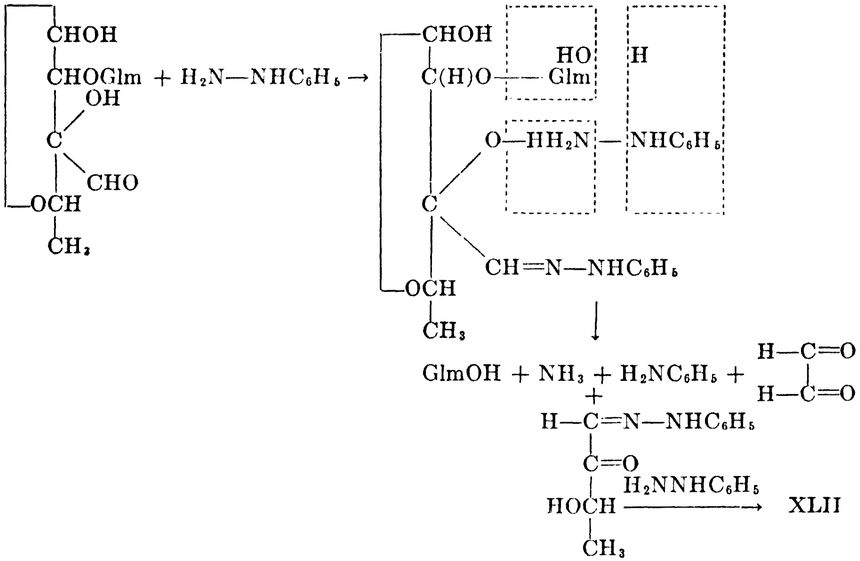
(88) J. Fried, Doris E. Walz and O. Wintersteiner, *J. Am. Chem. Soc.*, **68**, 2746 (1946).

(89) C. S. Hudson, *J. Am. Chem. Soc.*, **32**, 338 (1910); **61**, 1525 (1939).

(90) For this nomenclature, see J. C. Sowden, *J. Am. Chem. Soc.*, **69**, 1047 (1947) and M. L. Wolfrom and A. Thompson, *ibid.*, **67**, 1793 (1945).

(91) M. L. Wolfrom, S. W. Waisbrot and R. L. Brown, *J. Am. Chem. Soc.*, **64**, 2329 (1942).

that dihydrostreptobiosamine failed to yield the osazone under similar conditions. On this basis, the formation of the osazone from streptobiosamine would take place in the general manner indicated below.


XLII

Acetylation of dihydrostreptobiosamine hydrochloride with acetic anhydride and pyridine yielded as the main product a hexaacetate, $[\alpha]_D$ −108°, designated α, and smaller amounts of two other hexaacetates designated β, $[\alpha]_D$ −36.2°, and γ, $[\alpha]_D$ −175°.[92] From the optical data it would appear that the α and β hexaacetates are anomers differing from each other by stereoisomerism at C1 of the dihydrostreptose moiety and that they correspond sterically to the α and β forms of methyl pentaacetyldihydro-L-streptobiosaminide (see page 357), the specific rotations of which are $[\alpha]_D$ −117° and −34°, respectively. For these two isomers the ring structure in the streptose portion would be 1,4-furanose, as in structure XXXV. Since streptose was shown to be an L sugar, this assignment of prefixes is in accord with the established conventions for designating anomers on the basis of their specific rotations. Hydrolysis of the α and β hexaacetates with boiling water was shown[92] to eliminate the acetyl group situated at C1 of the dihydrostreptose moiety. The amorphous products obtained had properties similar to those of the amorphous product formed by the hydrolytic removal, with mercuric chloride, of the ethylmercapto group in ethyl dihydrothiostreptobiosaminide pentaacetate.[92]

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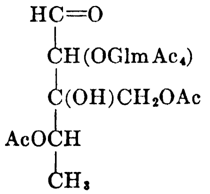
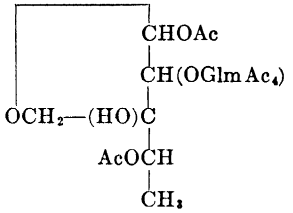
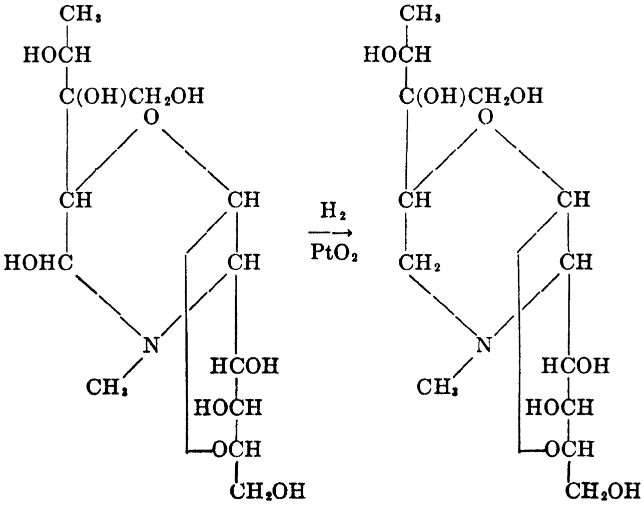
(92) H. E. Stavely, O. Wintersteiner, J. Fried, H. L. White and M. Moore, *J. Am. Chem. Soc.*, **69**, 2742 (1947).

The highly levorotatory γ-hexaacetate would have to be a structural isomer of the two anomeric α and β hexaacetates. It was pointed out that the aldehydo structure XLIII was improbable from the mode of its formation; moreover it was excluded by the fact that the compound failed to give rise, on treatment with thiosemicarbazide, to the ultraviolet absorption band at 2700 Å characteristic of thiosemicarbazones.[60] Since ring isomerism in the N-methyl-L-glucosamine portion or a 1,3-orthoacetate structure did not seem probable, the 1,6-furanose structure (XLIV) was tentatively proposed for this compound.


XLIII


XLIV

Hydrogenation of dihydrostreptobiosamine hydrochloride and of streptobiosamine hydrochloride with platinum oxide catalyst resulted in the absorption of one and two moles of hydrogen, respectively. Acetylation of the products yielded in each case a crystalline compound which, however, was not the expected heptaacetyltetrahydrostreptobiosamine, but a base hydrochloride of the composition $C_{23}H_{20}O_8N(CH_3CO)_5{\cdot}HCl{\cdot}C_2H_5OH$. The mole of ethanol, which could not be removed by drying

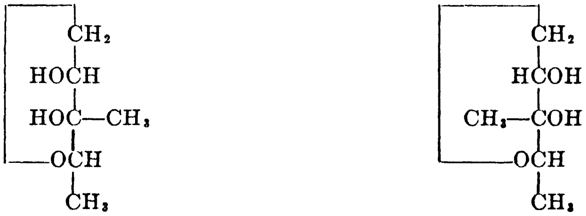

XLV XLVI

at 80°, was probably not an integral part of the molecule but was probably derived from the solvent used in the crystallization. This substance was non-reducing to Fehling solution, retained its basic properties, and could not have a N-acetyl group introduced on acetylation. It was therefore concluded that the reduction yielded the morpholine structure XLVI, designated (after acetylation) pentaacetyltetrahydroanhydrostreptobiosamine.

Drastic acid hydrolysis, under conditions which were known to cleave the glycosidic linkage between the two moieties of dihydrostreptobiosamine failed to hydrolyze the tetrahydroanhydrostreptobiosamine. This unusual resistance to acid was likened to the similar stability toward acid hydrolysis exhibited by di-D-fructopyranose anhydride[93] and di-D-fructofuranose anhydride,[94] in which the reducing groups of the two D-fructose residues are parts of a dioxan ring as well as of the pyranose or furanose rings.

Acetonation of methyl dihydrostreptobiosaminide by the method of Dangschat and Fischer[95] followed by acetylation and chromatographic separation, afforded a crystalline compound.[92] The analytical data, though not quite conclusive, fit best for a dimer, $C_{42}H_{64}O_{21}N_2$, containing two isopropylidene groups and five acetyl groups, one of which was bound to nitrogen.

Didesoxydihydrostreptose formed an acidic complex with boric acid.[84] In accordance with the extensive work of Böeseken and his students, this is indicative of a configurationally *cis* α-glycol grouping and the configuration of didesoxydihydrostreptose would be one of the two formulas shown (XLVII or XLVIII). Streptose itself would then have either the configurational system of L-ribose or L-lyxose and would



XLVII XLVIII

Didesoxydihydrostreptose
(possible configurational formulas)

be either 3-C-formyl-5-desoxy-L-ribose (XLIX) or 3-C-formyl-5-desoxy-L-lyxose (L).

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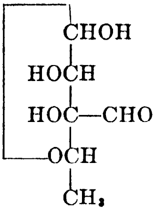
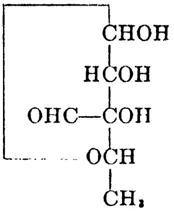
[93] H. H. Schlubach and C. Behre, *Ann.*, **508**, 16 (1934); see also Emma J. McDonald, *Advances in Carbohydrate Chem.*, **2**, 253 (1946).

[94] W. N. Haworth and H. R. L. Streight, *Helv. Chim. Acta*, **15**, 693 (1932).

[95] Gerda Dangschat and H. O. L. Fischer, *Naturwissenschaften*, **30**, 146 (1942).



XLIX

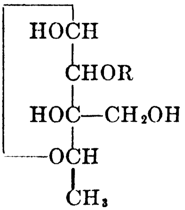


L

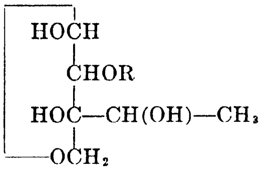
Streptose
(Possible configurational formulas)

A number of points regarding the chemical behavior of the streptose moiety are anomalous and will bear comment. Although a free carbonyl group seems established, streptomycin itself shows no carbonyl absorption in the ultraviolet region. Derivatives of the acyclic or aldehydo structure have hitherto exhibited such absorption.[96,97] Oxidation of this aldehyde substituent to the carboxyl stage or its reduction to either the carbinol or hydrocarbon did not affect the resistance of the tertiary hydroxyl toward acetylation. However, conversion of both aldehyde functions in streptose to the carboxyl stage of oxidation made the tertiary hydroxyl easily esterifiable.[86]

The lactol ring structure of streptose as combined in streptomycin (XXXIX) is of the furanose type. Hitherto, furanosides have been found to be so readily hydrolyzed by acids that this characteristic has been employed to establish the presence of such a ring. Yet in streptobiosamine the furanose ring of the streptose portion is so stable[47] that it does not even open in concentrated hydrochloric acid to yield a thioacetal (see, however, page 381). It is of further theorical interest to note that, when two possible furanose structures of the forms LI and LII are possible in the same compound, the structure LI seems to be the more preferred and for this structure the α-form appears to predominate.



LI



LII

Probably the chief stumbling-block encountered in establishing the structure of streptomycin was the extraordinary resistance toward acid

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(96) H. Hudson, M. L. Wolfrom and T. M. Lowry, *J. Chem. Soc.*, 1179 (1933).

(97) W. C. G. Baldwin, M. L. Wolfrom and T. M. Lowry, *J. Chem. Soc.*, 696 (1935).

hydrolysis of the glycosidic bond between the N-methyl-L-glucosamine and streptose portions. This enhanced stability seems to be common to all the glycosidic bonds of 2-desoxy-2-aminosugars. An explanation[86c] for this phenomenon seems evident when it is remembered that the $—NR_3^+$ group is highly electronegative. The stability of hydrates and hemiacetals formed at a carbonyl attached to a strongly electronegative group is well known, as is also the stabilizing effect on acetals brought about by strongly electron-attracting substituents. An example of the latter is the marked resistance to acid hydrolysis exhibited by dichloral-D-glucose (di(2,2,2-trichloroethylidene)-D-glucose).[97a]

4. *Maltol Formation*

In order to establish definitely the structure of streptomycin, it was required to show that no carbon to carbon rearrangement occurred during the acid degradation of streptomycin to the various streptobiosamine derivatives. The formation of maltol (XIV, page 357) in 17% yield[57] from methyl streptobiosaminide dimethyl acetal provided some evidence that the streptose portion in this derivative was of identical structure with that in streptomycin, which likewise yielded maltol with alkali.[80] However, maltol possesses a linear carbon skeleton, while the streptose portion of the various streptobiosamine derivatives was shown to contain a branched carbon skeleton. It was therefore evident that a carbon to carbon rearrangement occurred during the formation of maltol by alkali.

Some interesting experimental data regarding the nature of this rearrangement exist. A free carbonyl was the first essential, since the alkaline degradation of either dihydrostreptomycin[52,76] or streptomycinic acid[52] did not lead to maltol. The other requirement was that the second aldehyde group be glycosidically combined. Thus tetraacetylstreptobiosamine and N-acetylstreptobiosamine gave no maltol on treatment with alkali but methyl N-acetylstreptobiosaminide did.[86] Folkers and coworkers[86] prepared methyl N-acetylstreptobiosaminide in solution by treating tetraacetylstreptobiosamine (obtained by the demercaptalation of its crystalline tri(ethylmercapto) derivative) with hydroxylamine and then with methanolic hydrogen chloride followed by deoximation[98] with nitrous acid. Fried and Wintersteiner[52] prepared methyl streptobiosaminide hydrochloride by the selective hydrolysis of the non-glycosidic acetal group in methyl streptobiosaminide dimethyl acetal (see page 365).

Fried and Wintersteiner[52] have pointed out the analogy between the formation of maltol from streptomycin and the transformation of tetra-

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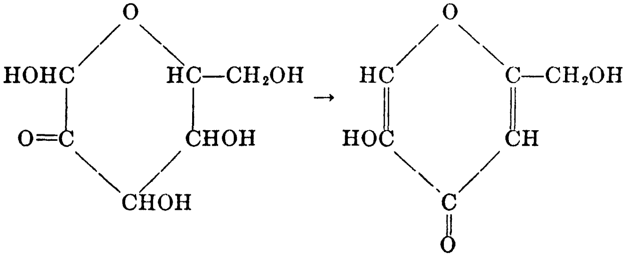
(97a) J. H. Ross and J. M. Payne, *J. Am. Chem. Soc.*, **45**, 2363 (1923).

(98) M. L. Wolfrom, L. W. Georges and S. Soltzberg, *J. Am. Chem. Soc.*, **56**, 1794 (1934).

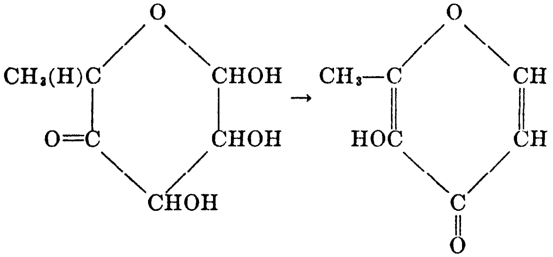
acetyl-D-glucosone hydrate,[99,100] as well as the corresponding D-galactose derivative,[101] to diacetylkojic acid by pyridine and acetic anhydride at 0°. Stacey and Turton[102] have repeated and extended the observations of Maurer and coworkers. They have proposed a mechanism for this transformation, as has Isbell.[103]

Inspection of the molecular structures for D-glucosone (LIII) and kojic acid (LIV) shows that the carbon atom which contained the ketonic carbonyl group in the D-glucosone molecule contains the enolic hydroxyl group in kojic acid.



LIII LIV
Kojic acid

If this condition be carried over to the formation of maltol, then this compound would be expected to arise from a molecule of structure LV.



LV Maltol

The formation of LV from streptose is not beyond expectation when it is recalled that α-hydroxyisobutyraldehyde can rearrange, under the influence of weak alkali or weak acid to acetoin.[104] The extensive investigation of this very interesting rearrangement, carried out by Danilov

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(99) K. Maurer, *Ber.*, **63**, 25 (1930).

(100) K. Maurer and W. Petsch, *Ber.*, **64**, 2011 (1931).

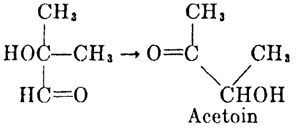
(101) K. Maurer and A. Müller, *Ber.*, **63**, 2069 (1930).

(102) M. Stacey and Lilian M. Turton, *J. Chem. Soc.*, 661 (1946).

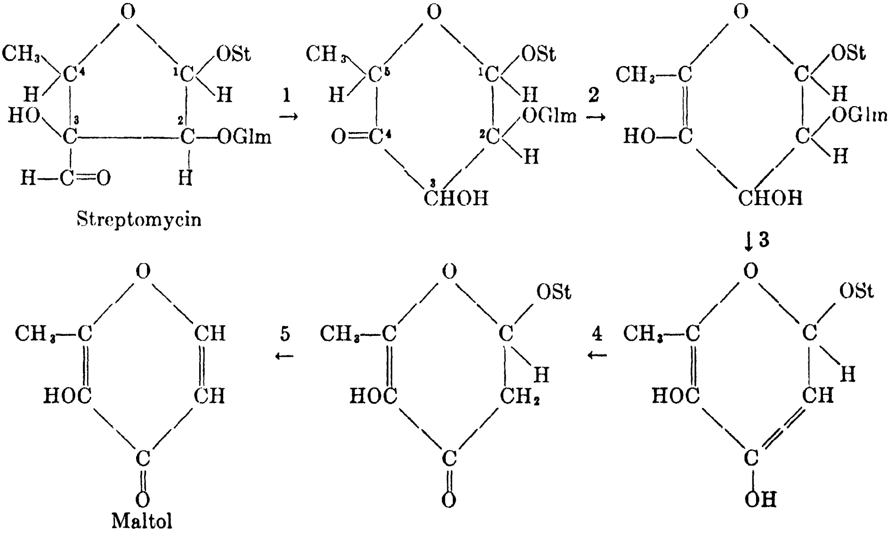
(103) H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

(104) S. Danilov and E. Venus-Danilova, *Ber.*, **67**, 24 (1934); *J. Gen. Chem.* (U. S. S. R.), **3**, 559 (1933).

and Venus-Danilova, does not appear to be well known. Although they devoted most of their attention to the rearrangement as catalyzed by a few drops of sulfuric acid, they showed in many cases that the isomeric transformation also proceeds in basic medium. The rearrangement of tertiary α-hydroxy aldehydes to α-hydroxy ketones was established for the following compounds: α,α-diphenylglycolaldehyde to benzoin,[104a] α,α-dicyclohexylglycolaldehyde to 1,2-dicyclohexyl-2-hydroxyethanone[104b] and 1-hydroxycyclohexanecarboxaldehyde to α-hydroxysuberone.[104c] The isomeric transformation of 1-hydroxycyclopentanecarboxaldehyde to 2-hydroxycyclohexanone[104d] is especially pertinent. This ring expansion took place in either alkaline or acid medium and it parallels very nicely the proposed ring expansion for streptose.



The following reactions are therefore suggested[86c] for the degradation of streptomycin to maltol by alkali.



(104*a*) S. Danilov, *Ber.*, **60**, 2390 (1927).

(104*b*) S. Danilov and E. Venus-Danilova, *Ber.*, **62**, 2653 (1929).

(104*c*) E. D. Venus-Danilova and V. F. Kazimirova, *J. Gen. Chem.* (U. S. S. R.), **7**, 2639 (1937); *C. A.*, **32**, 2099 (1938).

(104*d*) E. D. Venus-Danilova, *J. Gen. Chem.* (U. S. S. R.), **6**, 1784 (1936); *C. A.*, **31**, 4281 (1937).

In step 1, it is reasonable to assume that linkage C2 to C3 of the streptose portion of streptomycin would be the one to rupture since Fried, Walz and Wintersteiner[88] showed this link to be highly labile by being the one which was broken under the influence of phenylhydrazine. For step 2, enediol formation would be expected to take place between carbon atoms 4 and 5 because of the presence of the methyl group at the C5 position. It is well known that the ease of aldol condensation at the α-position of an aldehyde increases with increasing aliphatic substitution at this position; thus, the readiness for aldol condensation is $(CH_3)_2CH—CHO > CH_3CH_2CHO > CH_3CHO$. The formation of an enol intermediate is believed necessary for this reaction. Step 3 is assumed on the basis that the lability of the C2 to C3 bond in the streptose portion of streptomycin was in part due to the creation of high electron density and polarizability at the C2 atom because of the N-methyl-L-glucosamine substituent. If this be true, then the elimination of N-methyl-L-glucosamine, as represented in step 3, would be preferred over the elimination of water. The general tendency of systems to create conjugated linkages and thereby gain resonance energy would also favor this step. The lability of the C2 to C3 bond in the streptose portion of streptomycin may be attributed also to the fact that this link is situated between two carbon atoms one of which is linked to an aldehydic carbonyl group and the other to a lactol carbon atom. This kind of bond activation is well established for the A to B link in molecules of the general formula A:B:C::D. Step 5 would be essentially a dehydration such as is common in the formation of γ-pyrones. It is thus evident that the isolation of maltol as a product of the alkaline degradation of streptomycin does not introduce doubt into the validity of the structure for streptose as presented in formula XXXIX (page 366). The fact that a glycoside of streptobiosamine is required for the formation of maltol finds ready explanation when it is considered that, if the other aldehyde position in streptose is free, enediol formation could begin from this position and upset the mechanism which leads to maltol.

IV. THE GLYCOSIDIC BONDS IN STREPTOMYCIN

Hudson's rules of isorotation[105,106] allow the ready calculation of the rotation contribution made by the lactol or glycosidic carbon in a sugar or sugar derivative. Although these relationships are not mathematically exact, they have been shown to hold sufficiently closely in the carbohydrate group to justify their use in allocating anomeric configurations. Two anomeric forms of pentaacetyl-N-methyl-L-glucosamine are known. The α form was recorded by Folkers and coworkers[65] and the β form was

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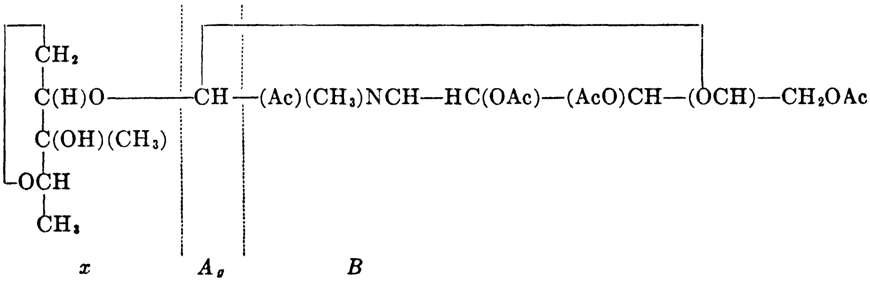
[105] C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909); see also page 15.

[106] C. S. Hudson, *Sci. Papers Bur. Standards*, **21**, No. 533, 241 (1926).

obtained by Wolfrom and Thompson[69] and by Stavely, Wintersteiner, Fried, White and Moore.[92] According to Hudson's rules of isorotation, $[M]_\alpha = A + B$ and $[M]_\beta = -A + B$, wherein $[M]$ is the molecular rotation of the anomer designated in the subscript, A is the rotation contribution of the lactol carbon and B is the rotation contribution of the remainder of the molecule. Solving[87] for A, the value $-17{,}400$ (5892.5 Å, chloroform solvent) was found, as compared with $+18{,}000$ for A in the analogous derivatives of D-glucosamine and $+19{,}100$ for those of D-glucose. Thus the introduction of the N-methylamino group into the glucose molecule does not significantly alter this constant. The value of B was then $-21{,}150$. Now the molecular rotation of tetraacetyldidesoxydihydro-L-streptobiosamine ($[\alpha]_D$ − 86°, mol. wt. 475.5) may be expressed as $[M]_D = 475.5 \times -86 = -40{,}900 = A_g + B + x$, where A_g is the rotatory contribution of the lactol carbon, B that of the remainder of the N-methyl-L-glucosamine portion (see LVI) and x that of the optically active didesoxydihydrostreptose moiety. This equation becomes



LVI

$A_g = -16{,}750 - x$. The value of x is not known, but we assume that it is not greatly different from that of didesoxydihydrostreptose, which is $+4{,}200$[84]; in such case $A_g = -20{,}950$. Since the assumed rotation contribution of x is low it does not greatly affect the result. Table I shows the values of A calculated for a number of glucose derivatives in which A lies between two optically active portions. The value of A found for the acetylated streptobiosamine glycoside from streptomycin is sufficiently close to the values of Table I to make it highly probably that the glycosidic union is α-L. Numerical agreement is not required. The problem is merely to determine whether the experimental value most closely approximates $+25{,}000$ (β-L) or $-25{,}000$ (α-L). It is of interest to note that the order of groups about the lactol carbon for an α-L configuration is the same as that in a β-D configuration.

Carter, Loo and Skell[107] oxidized streptidine with periodic acid and

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[107] H. E. Carter, Y. H. Loo and P. S. Skell, *J. Biol. Chem.*, **168**, 401 (1947).

TABLE I

Rotatory Value of a D-Glucose Lactol Bond (A) Situated between Two Optically Active Constituents

Substance	$[M]^{20}_{D}$ (*chloroform*[a])	*A*
α-D-Maltose octaacetate	+83,000°	27,600
α-D-Cellobiose octaacetate	+27,800	
β-D-Maltose octaacetate	+42,500	26,200
β-D-Cellobiose octaacetate	−9,900	
Methyl heptaacetyl-β-D-maltoside	+34,900	25,700
Methyl heptaacetyl-β-D-cellobioside	−16,500	
Heptaacetyl-α-D-maltosyl chloride	+104,100	28,150
Heptaacetyl-α-D-cellobiosyl chloride	+47,800	
levo-Menthyl α-D-glucopyranoside	+20,400[b]	25,100
levo-Menthyl β-D-glucopyranoside	−29,800[b]	

[a] Data from ref. 106.
[b] Measured in ethanol solution.

noted that in the reaction mixture the Sakaguchi test[48] disappeared and a positive Pauly test[108] was obtained. This was interpreted as probably indicating the presence of an imidazole derivative formed by ring closure from the guanidinoglutaraldehyde resulting from the periodate oxidation of streptidine. A model substance, 1-guanidino-2,3-dihydroxypropane, behaved similarly. When streptomycin was treated for seventy-two hours with an excess of periodate at *p*H 5–6, the Sakaguchi test was still obtainable from the oxidation mixture and the Pauly test was negative. Therefore, the streptobiosamine is apparently attached to streptidine in such a position as to interfere with the oxidation of the streptidine ring by periodate. Therefore attachment of the streptidine portion at the upper position in formula I is indicated. The authors[107] state that the position adjacent to this cannot be disregarded entirely since a 1,2-glycol grouping in 1,2,3,4-tetraacetylinositol has been reported[95] as resistant to periodate oxidation. A similar effect has been noted[109] for the glycol grouping in D-glucosan <1,4>β<1,6> (see also page 51). Folkers and coworkers[109a] obtained a benzoylated streptomycin which was degraded to a crystalline heptabenzoylstreptidine. Treatment of this compound with methanesulfonyl chloride (mesyl chloride) and pyridine yielded

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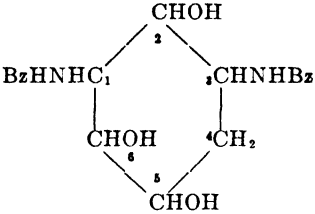
(108) H. Pauly, *Z. physiol. Chem.*, **42,** 508 (1904); **94,** 426 (1915). See K. Inouye, *ibid.*, **83,** 79 (1913).

(109) R. J. Dimler, H. A. Davis and G. E. Hilbert, *J. Am. Chem. Soc.*, **68,** 1377 (1946).

(109*a*) F. A. Kuehl, Jr., R. L. Peck, C. E. Hoffhine, Jr., Elizabeth W. Peel and K. Folkers, *J. Am. Chem. Soc.*, **69,** 1234 (1947).

mesylheptabenzoylstreptidine. The ability of sodium iodide in acetone solution to replace secondary mesyloxy groups by iodine[109b] was then utilized to provide an iodoheptabenzoyl streptidine which was reduced to heptabenzoyldesoxystreptidine and this latter compound afforded a N,N′-dibenzoyldesoxystreptamine.

Treatment of N,N′-dibenzoyldesoxystreptamine and N,N′-dibenzoylstreptamine under comparable conditions with periodate led to the consumption of one and two moles of periodate, respectively. These data were interpreted to show that the structure of the N,N′-dibenzoyldesoxystreptamine was LVII and thus the streptobiosamine portion in streptomycin was attached to the C4 position of the streptidine moiety. However, this proof is not definitive since it is well established that partially acylated polyhydroxy compounds are subject to acyl wandering. It appears probable that methylation techniques will be required for a rigorous proof of the point of attachment of streptobiosamine to streptidine.



LVII

If the symmetrical point of attachment be accepted, it is to be noted (see formula I) that the optical inactivity of the streptidine moiety in streptomycin would remain unaltered. An unsymmetrical substitution such as would result if the guanidino group were in the 2,4 positions, would render the streptidine portion potentially optically active. Accepting the optically inactive nature of the streptidine aglycon, the determination of the configuration of this glycosidic linkage becomes a relatively simple matter.[87] Methyl pentaacetyldihydro-L-streptobiosaminide has been isolated in two anomeric forms (see Table IV). Their molecular rotations in chloroform and at 5892.5 Å are $(-117 \times 563 = -65{,}900)$ and $(-34 \times 563 = -19{,}100)$, respectively. One-half of the sum of these molecular rotations gives a value of B' for the pentaacetyldihydrostreptobiosaminide entity in dodecaacetyldihydrostreptomycin of $-42{,}500$. The value $-42{,}600$ is calculable from the analogous anomeric forms[92] of hexaacetyldihydrostreptobiosamine. The molecular rotation, $[M]_D$ for dodecaacetyldihydrostreptomycin[87] was found to be $(-67 \times 1088 = -72{,}900)$ for a chloroform solution at room temperature.

(109b) B. Helferich and A. Gnüchtel, *Ber.*, **71**, 712 (1938).

Thus $A_s + B' = -72{,}900$ and since $B' = -42{,}500$, $A_s = -30{,}400$. This value is sufficiently close to those of Table I to indicate that in all probability this linkage is of the α-L variety. The dodecaacetyldihydrostreptomycin was amorphous but was prepared through crystalline intermediates and was purified by chromatographic procedures.

V. CHEMICAL ASSAYS

Now that the chemical nature of streptomycin has been elaborated, it will be pertinent to note some of the chemical assay procedures which depend upon the groups present. Levy, Schwed and Sackett[110] have described a rapid polarographic method for the assay of streptomycin in solutions of high and low purity. Boxer and coworkers[111] have determined streptomycin, in clinical samples and in broth, by conversion of the streptomycin to maltol by alkaline degradation and subsequent utilization of the color reactions of maltol with ferric ions or with the phenol reagent of Folin for photometric determination of streptomycin. An assay involving the liberation of maltol under standard conditions and the measurement of its ultraviolet absorption at 3250 Å has also been reported.[112] Scudi, Boxer and Jelinek[113] observed that streptomycin gave a color test on substituting sodium hydroxide for the sodium carbonate in the Elson-Morgan assay[114] for glucosamine. The N-methyl-L-glucosamine moiety of streptomycin was shown to be responsible for the color formation. The method gave useful results on highly purified samples of streptomycin but as the purity of the samples decreased the results became high. It was pointed out[47] that an approximately linear relationship exists between the optical rotation and the bioassay of streptomycin preparations of 500 units per milligram or higher activity. Boxer and Jelinek[115] described a method for the estimation of streptomycin in blood and spinal fluid. The procedure was based upon the interaction of the free aldehyde group of streptomycin with fluorescent 9-hydrazinoacridine and the subsequent fluorometric determination of the substituted hydrazone so formed. A related procedure[116] for the

(110) G. B. Levy, P. Schwed and J. W. Sackett, *J. Am. Chem. Soc.*, **68**, 528 (1946).

(111) G. E. Boxer and Viola C. Jelinek, Abstracts of Papers, 110th Meeting, Am. Chem. Soc., Chicago, Illinois, September 9–13, p. 13B (1946); G. E. Boxer, Viola C. Jelinek and Patricia M. Leghorn, *J. Biol. Chem.*, **169**, 153 (1947).

(112) J. Fried, Nettie H. Coy and R. Donovick, cited in *J. Am. Chem. Soc.*, **69**, 83 (1947), footnote 23; J. Fried and E. Titus, *ibid.*, *in press* (1948).

(113) J. V. Scudi, G. E. Boxer and Viola C. Jelinek, *Science*, **104**, 486 (1946).

(114) L. A. Elson and W. T. Morgan, *Biochem. J.*, **27**, 1824 (1933).

(115) G. E. Boxer and Viola C. Jelinek, *Federation Proc.*, **6**, 241 (1947); *J. Biol. Chem.*, **170**, 491 (1947).

(116) E. K. Marshall, Jr., K. C. Blanchard and E. L. Buhle, *J. Pharmacol. Exptl. Therap.*, **90**, 367 (1947).

estimation of streptomycin in body fluids depends upon the colorimetric assay of the derivative formed with the colored 4-[4-(*p*-chlorophenylazo)-1-naphthyl] semicarbazide.

VI. STREPTOMYCIN B

Examination of streptomycin concentrates for homogeneity by the Craig technique of counter-current distribution[117] indicated the presence of another structurally related substance.[118] The application of the chromatographic method for the purification of streptomycin, described by Carter and coworkers,[36] yielded, in addition to highly active fractions of streptomycin, more firmly held fractions of low antibiotic activity. However, on treating these fractions with alkali a much higher yield of maltol was obtained than was expected on the basis of the bioassay. Following these observations, there was isolated,[119] as the crystalline reineckate, a substance designated streptomycin B. The reineckate was decomposed into a white, amorphous hydrochloride whose specific rotation in water, $[\alpha]_D$ $-47°$, was very appreciably less levorotatory than that of streptomycin trihydrochloride, $-87°$.[37] The substance did not form a crystalline calcium chloride complex.[120] Tested against eight strains or species of bacteria, it was found to be less active, on a weight basis, than streptomycin.[121] Its toxicity in mice was similar to that of streptomycin.[121]

Streptomycin B was inactivated by carbonyl reagents. On catalytic hydrogenation it absorbed one mole of hydrogen to form dihydrostreptomycin B. The hydrogenation did not cause any loss in activity. The activity of dihydrostreptomycin B was not impaired by carbonyl reagents and it did not yield maltol on treatment with alkali.

Both methanolysis and mercaptolysis of streptomycin B yielded known derivatives of streptidine, streptobiosamine and D-mannose, while dihydrostreptomycin B yielded derivatives of streptidine, dihydrostreptobiosamine and D-mannose.[122] The derivatives of D-mannose which were obtained are listed in Table II. These results, together with the analytical data for the hydrochloride and reineckate of streptomycin B, established the composition of the free base as $C_{27}H_{49}O_{17}N_7$ and showed that the substance exists as glycosidically linked streptidine,

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[117] L. C. Craig, *J. Biol. Chem.*, **155**, 519 (1944); L. C. Craig, C. Golumbic, H. Mighton and E. Titus, *ibid.*, **161**, 321 (1945).

[118] E. Titus and J. Fried, *J. Biol. Chem.*, **168**, 393 (1947).

[119] J. Fried and E. Titus, *J. Biol. Chem.*, **168**, 391 (1947).

[120] J. Fried, cited in reference 121.

[121] G. Rake, Clara M. McKee, F. E. Pansy and R. Donovick, *Proc. Soc. Exptl. Biol. Med.*, **65**, 107 (1947).

[122] J. Fried and H. E. Stavely, *J. Am. Chem. Soc.*, **69**, 1549 (1947).

L-streptobiosamine, and D-mannose. It is not known whether streptomycin B is a streptomycin glycoside of D-mannose or whether the D-mannose forms a central moiety.

The isolation by these workers of acetylated thioglycosides of D-mannose (see Table II) under mercaptolysis conditions (followed by acetylation) wherein the acetylated thioacetal would be expected,[123] is unusual and would indicate that the thioacetal was formed and then underwent ring closure. It is then very probable[86c] that the same process may occur in the mercaptolysis of streptomycin with the thioacetal being first formed at C1 of the streptose portion and subsequently undergoing ring closure to the thioglycoside. Thus the ring found in the streptobiosamine derivatives isolated need not necessarily have been the one originally present in streptomycin (see page 371).

TABLE II

D-*Mannose Derivatives from Streptomycin B*[122]

Substance	*M.P., °C.*	$[\alpha]_D$, *chloroform*
Methyl tetraacetyl-α-D-mannopyranoside	65–66	+49°
Methyl tetraacetyl-β-D-mannopyranoside	160–161	−50
Ethyl tetraacetylthio-α-D-mannoside	105–106	+94
Ethyl tetraacetylthio-β-D-mannoside	161–162	−67

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(123) See M. L. Wolfrom and J. V. Karabinos, *J. Am. Chem. Soc.*, **67**, 500 (1945).

VII. TABLES OF PROPERTIES OF SOME STREPTOMYCIN AND RELATED DERIVATIVES

TABLE III

Products Obtained in the Preparation of N-Methyl-L-glucosamine

Substance	*M.p., °C.*	$[\alpha]_D$[a]	*Rotation solvent*	*Reference*
N-Methyl-L-arabinosylamine	118–120	+43 → +51°	H_2O	73, 74
N-Methyl-L-glucosaminic acid nitrile	113	−17.5 → −21 → −8.3	H_2O	73, 74
N-Methyl-L-glucosaminic acid nitrile hydrochloride	138–140 (dec.)	−28.5 → +54.2 → +17.8	H_2O	69
Pentaacetyl-N-methyl-L-glucosaminic acid nitrile	132–134	−38	$CHCl_3$	73, 74
N-Methyl-L-glucosaminic acid	236 (dec.)	−4.6	H_2O	73, 74
		−3.0 → −9.1	HCl (2.5%)	74
	231–232 (dec.)	−5 → −8	HCl (0.6 *N*)	65
N-Methyl-D-glucosaminic acid	230 (dec.)	+4.0 → +7.5	HCl (2.5%)	124
N-Methyl-L-glucosaminic acid hydrochloride	136–137	−4.7	H_2O	69
N-Methyl-L-glucosamine	130–132 (dec.)	−64	H_2O	69
Pentaacetyl-N-methyl-α-L-glucosamine	161–162	−100	$CHCl_3$	65
	158.5–159.5	−102	$CHCl_3$	69
Pentaacetyl-N-methyl-β-L glucosamine	153–153.5	−16.5	$CHCl_3$	69
	151–152	−14.6	$CHCl_3$	92
Pentaacetyl-N-methyl-α-D-glucosamine	160.5–161.5	+101	$CHCl_3$	65
	158.5–159.5	+102	$CHCl_3$	69
Pentaacetyl-N-methyl-α-D,L-glucosamine	130–131	0	$CHCl_3$	69
N-Acetyl-N-methyl-L-glucosamine	165–166	−51	H_2O	65
N-Methyl-α-L-glucosamine hydrochloride	160–162	−106 → −89	H_2O	69
	168–170 (dec.)	−105 → −87	H_2O	65
N-Methyl-α-D-glucosamine hydrochloride	164–166 (dec.)	+104 → +89	H_2O	65
L-Glucosamine hydrochloride	—	−73	H_2O	65
Pentaacetyl-N-methyl-L-mannosaminic acid nitrile	112–113.5	−27.5	$CHCl_3$	69
N-Methyl-L-mannosaminic acid	195–197 (dec.)	−6.7	H_2O	69
		0 → −32	HCl (2.5%)	

[a] Measured at room temperature at $c < 5$.

(124) E. Votoček and R. Lukeš, *Collection Czechoslov. Chem. Communs.*, **7**, 424 (1935); *Chem. Listy*, **29**, 308 (1935).

TABLE IV

Acetyl Derivatives of Streptobiosamine

Substance	*M.p., °C.*	$[\alpha]_D$[a] *(chloroform)*	*Reference*
Partially acetylated derivatives			
Methyl tetraacetyl-α-L-streptobiosaminide dimethyl acetal	124.5–126	−124°	2, 57
Methyl tetraacetyl-β-L-streptobiosaminide dimethyl acetal	118.5–119.5	−45	87
Methyl tetraacetyl-L-streptobiosaminidic acid methyl ester	139–140	−134	52
Ethyl tetraacetyl-α-L-thiostreptobiosaminide diethyl thioacetal	80.5–81 81–82	−192 −178	47 3
Ethyl tetraacetyl-β-L-thiostreptobiosaminide diethyl thioacetal	111–111.5	−29	47
Tetraacetyldidesoxydihydro-L-streptobiosamine	159–160 158.5–159	−85 −86	3 47
Tetraacetyl-L-streptobiosamine	188–189	−78	86
Methyl pentaacetyldihydro-α-L-streptobiosaminide	198–198.5 193–194 194–195	−117 −120 −117	57 52 47
Methyl pentaacetyldihydro-β-L-streptobiosaminide	155.5–157	−34	57
Ethyl pentaacetyldihydro-α-L-thiostreptobiosaminide	116–116.5 115–116	−172 −165	82 92
Hexaacetyldihydro-α-L-streptobiosamine	143–144	−108	92
Hexaacetyldihydro-β-L-streptobiosamine	105–107	−36	92
Pentaacetyldihydro-α-L-streptobiosamine	140–142	−98	92
Hexaacetyldihydro-γ-streptobiosamine	135–136	−175	92
Pentaacetyldesoxydihydro-L-streptobiosamine	131	−87	82
Pentaacetyltetrahydroanhydro-L-streptobiosamine hydrochloride	154–155 (dec.)	+6.1 (H_2O)	92
Fully acetylated derivatives			
Pentaacetyl-L-streptobiosamic acid monolactone	162–163	−79 (CH_3OH)	86
Pentaacetyl-L-streptobiosamic acid methyl ester monolactone	162–163	—	86

Measured at room temperature at $c < 5$.

TABLE V

Derivatives of Streptose

Substance	*M.p., °C.*	$[\alpha]_D$[a]	*Rotation solvent*	*Reference*
L-Streptosonic acid monolactone	146–148	−37°	H_2O	86
Diacetyl-L-streptosonic acid monolactone	186–188	+26	CH_3OH	86
L-Streptosonic acid diamide	135–136	+14	H_2O	86
Didesoxydihydro-L-streptose	90–91	+32	$CHCl_3$	84
Di-*p*-nitrobenzoyldidesoxydihydro-L-streptose	141–142	—	—	84

[a] Measured at room temperature at $c < 5$.

ADDENDUM*

The Configuration of Streptidine

The configuration of streptidine has now been elucidated.[125] Partial deacetylation of pentaacetyl-D-glucosamine diethyl thioacetal yielded N-acetyl-D-glucosamine diethyl thioacetal which, analogous to the corresponding derivative of D-glucose,[126] gave ethyl 2-acetamido-2-desoxy-α-D-glucothiofuranoside on reaction with mercuric chloride in the presence of mercuric oxide. Oxidation of the thiofuranoside with lead tetraacetate yielded a sirupy aldehyde which was condensed immediately with nitromethane to produce ethyl 2-acetamido-6-nitro-2,6-didesoxy-α-D-glucothiofuranoside and ethyl 2-acetamido-6-nitro-2,6-didesoxy-β-L-idothiofuranoside. These two substances are the predictable reaction products but no evidence was obtained as to which of the two products (m. p. 112–113° and 190–193°, dec.) obtained had the D-glucose or the L-idose structure. The ethylmercapto group was hydrolyzed with mercuric chloride from the higher melting isomer and the resultant amorphous nitro sugar was cyclicized with dilute barium hydroxide. The amorphous barium salt so formed was hydrogenated in dilute acid solution with Raney nickel catalyst. Acetylation of the reduction mixture yielded two crystalline compounds which were characterized by their melting points and by x-ray diffraction diagrams. One of these was identical with hexaacetylstreptamine from streptomycin. Because of the known configuration of D-glucosamine, the known nature of the cyclization reaction[127] (see page 53), the fact that streptidine is a meso form, and the relationship of streptamine to streptidine (see page 350), it follows that streptidine is configurationally all-*trans* and that streptamine is the isomer corresponding to the last formula depicted on page 351.

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* Added during the reading of the proof.

(125) M. L. Wolfrom and S. M. Olin, Abstracts of Papers, 113th Meeting, Am. Chem. Soc., April 19–23 (1948).

(126) W. Schneider and Johanna Sepp, *Ber.*, **49**, 2054 (1916); J. W. Green and E. Pacsu, *J. Am. Chem. Soc.*, **59**, 1205 (1937); E. Pacsu and E. J. Wilson, Jr., *ibid.*, **61**, 1450 (1939); M. L. Wolfrom, S. W. Waisbrot, D. I. Weisblat and A. Thompson, *ibid.*, **66**, 2063 (1944).

(127) H. O. L. Fischer, *Harvey Lectures*, Ser. **40**, 156 (1945).

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ERRATA

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Page 3. It should have been stated that O. T. Schmidt and Catherina C. Weber Molster, *Ann.*, **515**, 43 (1934), have obtained evidence from rotatory relations for the full configurations of the two D-fructoheptonic acids. The oversight is regretted.—C. S. Hudson.

Page 11. The last paragraph can now be given final form by insertion of the following reference: Alice T. Merrill, W. D. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **69**, 70 (1947).—C. S. Hudson.

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Page 56, footnote 35. For "2058" read "2068."

Page 77, lines 7, 8, and 9 from bottom. Between "3,6-Anhydro-D-allose phenylosazone" and "2-methyl-" insert the heading "3,6-Anhydro-D-altrose."

Page 77, last two lines. Reference numbers 116 should be 37.

Page 167, footnote 16, and page 296 (author index). The name is E. G. Cox and the page number is 894.

Page 211, footnote 30; page 220, footnotes 65*a* and 65*b*; page 228, footnotes 103*a* and 103*b*. Change "Evelyn" to "E."

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